DEEP FREEZING OF OSMANABADI AND SIROHI BUCK SEMEN WITH
SPECIAL REFERENCE TO SPERM MEMBRANE INTEGRITY, DNA
DAMAGE AND FERTILIZING ABILITY

THESIS

Submitted

In partial fulfillment of the requirement for the Degree of

DOCTOR OF PHILOSOPHY

in

Animal Reproduction

BY

DAGLI NILESH RAMESHCHANDRA
(ENROLMENT NO : V/05/0273)

MAHARASHTRA ANIMAL AND FISHERY SCIENCES
UNIVERSITY

NAGPUR -440 001
(INDIA)

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DEPARTMENT OF ANIMAL REPRODUCTION, GYNAECOLOGY & OBSTETRICS
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MAHARASHTRA ANIMAL AND FISHERY SCIENCES UNIVERSITY
NAGPUR- 440 006
(INDIA)
2011
DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled "Deep-Freezing of Osmanabadi and Sirohi buck semen with special reference to sperm membrane integrity, DNA damage and fertilizing ability." or there of has not been submitted for any other degree or diploma of any other University, nor has the data been derived from any thesis/publication of any University or Scientific organisation. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

Date: 
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Enrolment No.: V/05/0273

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(Chairman Advisory Commitee)
Professor of Gynaecology
Declaration of Advisory Committee

Mr. Nilesh Rameshchandra Dagli has satisfactorily prosecuted his course of research for a period of not less than one semester and that the thesis entitled,

“Deep-Freezing of Osmanabadi and Sirohi buck semen with special reference to sperm membrane integrity, DNA damage and fertilizing ability.” Submitted by him is the result of research work sufficient to warrant its presentation to the examination in the subject of Animal Reproduction for the award of Ph.D degree by the Maharashtra and Animal Sciences University, Nagpur.

We also certify that the thesis or part of thereof has not been previously submitted by him for a degree of any other University.

Place: Mumbai
Date:

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Advisor/ Guide
Professor of Gynaecology

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   Deputy Director & Head of National Center for Pre-clinical Reproductive & Genetic Toxicology, NIRRH, Parel, Mumbai-12

iv) Dr. M.I. Baig
   Professor
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   Bombay Veterinary College

v) Dr. B.T. Deshmukh
   Professor
   Department of Physiology
   Bombay Veterinary College
This is to certify that the thesis entitled, "Deep-Freezing of Osmanabadi and Sirohi buck semen with special reference to sperm membrane integrity, DNA damage and fertilizing ability." submitted by Shri. Nilesh Rameshchandra Dagli to the Maharashtra and Animal Sciences University, in partial fulfillment of the requirement for the degree of Ph.D has been approved by the Student’s Advisory Committee after examination in collaboration with the External Examiner.

Name & Signature of External Examiner  Signature with seal  Name and signature
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Date: (Nilesh R. Dagli)
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Sr.NO</th>
<th>PARTICULARS</th>
<th>PAGE NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Introduction</td>
<td>01</td>
</tr>
<tr>
<td>II</td>
<td>Review of Literature</td>
<td>07</td>
</tr>
<tr>
<td>III</td>
<td>Material and Methods</td>
<td>55</td>
</tr>
<tr>
<td>IV</td>
<td>Results and Discussion</td>
<td>71</td>
</tr>
<tr>
<td>V</td>
<td>Summary and Conclusions</td>
<td>204</td>
</tr>
<tr>
<td>A</td>
<td>Bibliography</td>
<td>I</td>
</tr>
<tr>
<td>B</td>
<td>Thesis Abstract (English and Marathi)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Vita</td>
<td></td>
</tr>
</tbody>
</table>
# INDEX

<table>
<thead>
<tr>
<th>CHAPTER NO.</th>
<th>SUB-CHAPTER NO.</th>
<th>CHAPTER</th>
<th>PAGE NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td></td>
<td>INTRODUCTION</td>
<td>1 – 6</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td>REVIEW OF LITERATURE</td>
<td>7 – 54</td>
</tr>
<tr>
<td></td>
<td>2.1</td>
<td>Macroscopic evaluation of semen</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Volume of semen</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Colour and consistency of semen</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Density of semen</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hydrogen ion concentration (pH) of semen</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Resazurin test</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>Microscopic evaluation of semen</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>2.2.1</td>
<td>Mass activity</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>2.2.2</td>
<td>Sperm concentration</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>2.2.3</td>
<td>Sperm motility percentage</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>2.2.3a</td>
<td>Motility of sperm in neat Semen</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>2.2.3b</td>
<td>Pre/Post freeze motility of spermatozoa</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>2.2.4</td>
<td>Live sperm percentage</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>2.2.4a</td>
<td>Neat semen</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>2.2.4b</td>
<td>Pre/post freeze live and dead sperm percentage</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>2.2.5</td>
<td>Abnormal sperm percentage</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>2.2.5a</td>
<td>Neat semen</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>2.2.5b</td>
<td>Pre/Post freeze abnormal sperm percentage</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>2.3</td>
<td>Sperm function test</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>2.3.1</td>
<td>Plasma membrane integrity (Hypo-Osmotic Swelling Test)</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>2.3.2</td>
<td>Acrosome Integrity</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>2.3.3</td>
<td>DNA integrity test</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>2.3.3.1</td>
<td>DNA integrity by Acridine orange test</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>2.3.3.2</td>
<td>DNA integrity test by single gel electrophoresis (Comet Assay)</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>2.4</td>
<td>Enzyme leakage test</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>2.4.1</td>
<td>Transaminase (GOT/GPT)</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>2.4.2</td>
<td>Phosphatases (AKP/ACP)</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>2.4.3</td>
<td>Dehydrogenase (LDH)</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>Conception rates with frozen buck semen</td>
<td>51</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td>MATERIALS AND METHODS</td>
<td>55–72</td>
</tr>
<tr>
<td></td>
<td>3.1</td>
<td>Selection of animals</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>3.2</td>
<td>Sterilization of equipment and rubber material</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>3.3</td>
<td>Semen collection</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>3.4</td>
<td>Statistical analysis</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>Semen evaluation of Osmanabadi and Sirohi bucks</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>3.5.1</td>
<td>Macroscopic evaluation of semen</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>3.5.1.1</td>
<td>Ejaculate volume</td>
<td>57</td>
</tr>
<tr>
<td>Section</td>
<td>Description</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>3.5.1.2</td>
<td>Colour and Consistency</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>3.5.1.3</td>
<td>Density</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>3.5.1.4</td>
<td>Resazurin reduction test</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>3.5.2</td>
<td>Microscopic evaluation of semen</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>3.5.2.1</td>
<td>Mass activity</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>3.5.2.2</td>
<td>Initial motility</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>3.5.2.3</td>
<td>Sperm concentration per ml</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>3.5.2.4</td>
<td>Percentage of live spermatozoa</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>3.5.2.5</td>
<td>Percentage of abnormal spermatozoa</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>3.5.2.6</td>
<td>Hydrogen ion concentration (pH) of semen</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>3.5.3</td>
<td>Sperm function tests</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>3.5.3.1</td>
<td>Hypo-osmotic Swelling test (HOS test)</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>3.5.3.2</td>
<td>Acrosomal intactness of spermatozoa</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>3.5.3.3</td>
<td>Test for DNA damage</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>3.5.4</td>
<td>Enzyme leakage test</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>3.5.4.1</td>
<td>Estimation of GOT and GPT in seminal plasma</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>3.5.4.2</td>
<td>Estimation of AKP and ACP in seminal plasma</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>3.5.4.3</td>
<td>Estimation of LDH in seminal plasma</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>3.6</td>
<td>Dilution of semen</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>3.6.1</td>
<td>Dilutors used for semen preservation</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>3.6.2</td>
<td>Dilution rate and freezing of semen</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>3.7</td>
<td>Preservation of semen at sub-zero temperature</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>3.7.1</td>
<td>Printing of french medium straws</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>3.7.2</td>
<td>Filling and sealing of french medium straws</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>3.7.3</td>
<td>Freezing of straws</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>3.8</td>
<td>Evaluation of thawed semen</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>3.9</td>
<td>Study of conception rate</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>3.9.1</td>
<td>Thawing of semen</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>3.9.2</td>
<td>Artificial insemination</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>3.9.3</td>
<td>Pregnancy diagnosis</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION</td>
<td></td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>4.1</td>
<td>Macroscopic characteristics of neat semen of Osmanabadi and Sirohi bucks</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>4.1.1</td>
<td>Ejaculate volume</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>4.1.2</td>
<td>Colour, Consistency and Density of neat semen.</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>4.1.3</td>
<td>Hydrogen ion concentration (pH) of semen</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>4.1.4</td>
<td>Resazurin test</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>4.2</td>
<td>Microscopic evaluation of semen of Osmanabadi and Sirohi bucks</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>4.2.1</td>
<td>Mass activity</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>4.2.2</td>
<td>Sperm concentration (millions/ml)</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>4.2.3</td>
<td>Initial sperm motility percent in neat semen</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>4.2.4</td>
<td>Live sperm percent in neat semen</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>4.2.5</td>
<td>Abnormal sperm percentage in neat semen.</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>4.3</td>
<td>Evaluation of neat semen of Osmanabadi and Sirohi bucks using sperm function test</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>---------------------------------------------------------------------------------</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>4.3.1</td>
<td>Plasma membrane integrity (Hypo-Osmotic Swelling Test- HOST)</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>4.3.2</td>
<td>Acrosome integrity (Giemsa stain)</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>4.3.3</td>
<td>DNA damage</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>4.3.3.1</td>
<td>DNA damage (Acridine orange test)</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>4.3.3.2</td>
<td>DNA damage (single gel electrophoresis- Comet assay)</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>4.4</td>
<td>Enzymes in seminal plasma of Osmanabadi and Sirohi bucks</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>4.4.1</td>
<td>Transaminase (GOT and GPT)</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>4.4.2</td>
<td>Alkaline phosphatase (AKP) and Acid phosphatase (ACP)</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>4.4.3</td>
<td>Lactate Dehydrogenase (LDH)</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>Evaluation of semen quality of Osmanabadi and Sirohi bucks at different freezing stages in different dilutors</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>4.5.1</td>
<td>Sperm Motility</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>4.5.1.1</td>
<td>Sperm motility percentage at pre-freezing (5°C) stage</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>4.5.1.2</td>
<td>Sperm motility percentage at post-freezing (24 hours) stage</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td>4.5.1.3</td>
<td>Sperm motility percentage at post-freezing (72 hours) stage</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>4.5.1.4</td>
<td>Sperm motility percentage in TYG dilutor</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>4.5.1.5</td>
<td>Sperm motility percentage in TYE dilutor</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>4.5.1.6</td>
<td>Sperm motility percentage in TYD dilutor</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>4.5.2</td>
<td>Live sperm percentage</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>4.5.2.1</td>
<td>Live sperm percentage at pre-freezing (5°C) stage</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>4.5.2.2</td>
<td>Live sperm percentage at post-freezing (24 hours) stage</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>4.5.2.3</td>
<td>Live sperm percentage at post-freezing (72 hours) stage</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>4.5.2.4</td>
<td>Live sperm percentage TYG dilutor</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td>4.5.2.5</td>
<td>Live sperm percentage TYE dilutor</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>4.5.2.6</td>
<td>Live sperm percentage TYD dilutor</td>
<td>105</td>
<td></td>
</tr>
<tr>
<td>4.5.3</td>
<td>Abnormal sperm percentage</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>4.5.3.1</td>
<td>Abnormal sperm percentage at pre-freezing (5°C) stage</td>
<td>108</td>
<td></td>
</tr>
<tr>
<td>4.5.3.2</td>
<td>Abnormal sperm percentage at post-freezing (24 hours) stage</td>
<td>109</td>
<td></td>
</tr>
<tr>
<td>4.5.3.3</td>
<td>Abnormal sperm percentage at post-freezing (72 hours) stage</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>4.5.3.4</td>
<td>Abnormal sperm percentage in TYG dilutor</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>4.5.3.5</td>
<td>Abnormal sperm percentage in TYE dilutor</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>4.5.3.6</td>
<td>Abnormal sperm percentage in TYD dilutor</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>4.5.4</td>
<td>Plasma membrane integrity of spermatozoa (HOST)</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>4.5.4.1</td>
<td>Plasma membrane integrity of spermatozoa at pre-freezing (SOC) stage</td>
<td>116</td>
<td></td>
</tr>
<tr>
<td>4.5.4.2</td>
<td>Plasma membrane integrity of spermatozoa at post-freezing (24 hours) stage</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td>4.5.4.3</td>
<td>Plasma membrane integrity of spermatozoa at post-freezing (72 hours) stage</td>
<td>118</td>
<td></td>
</tr>
<tr>
<td>4.5.4.4</td>
<td>Plasma membrane integrity of spermatozoa in TYG dilutor</td>
<td>119</td>
<td></td>
</tr>
<tr>
<td>4.5.4.5</td>
<td>Plasma membrane integrity of spermatozoa in TYD dilutor</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>4.5.4.6</td>
<td>Plasma membrane integrity of spermatozoa in TYE dilutor</td>
<td>121</td>
<td></td>
</tr>
<tr>
<td>4.5.5</td>
<td>Acrosome intactness of spermatozoa (Giemsa stain)</td>
<td>123</td>
<td></td>
</tr>
<tr>
<td>4.5.5.1</td>
<td>Acrosome intactness of spermatozoa at pre-freezing (5°C) stage</td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>4.5.5.2</td>
<td>Acrosome intactness of spermatozoa at post-freezing (24 hours) stage</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td>4.5.5.3</td>
<td>Acrosome intactness of spermatozoa at post-freezing (72 hours) stage</td>
<td>126</td>
<td></td>
</tr>
<tr>
<td>4.5.5.4</td>
<td>Acrosome intactness of spermatozoa in TYG dilutor</td>
<td>126</td>
<td></td>
</tr>
<tr>
<td>4.5.5.5</td>
<td>Acrosome intactness of spermatozoa in TYD dilutor</td>
<td>127</td>
<td></td>
</tr>
<tr>
<td>4.5.5.6</td>
<td>Acrosome intactness of spermatozoa in TYE dilutor</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>4.5.6</td>
<td>DNA damaged of spermatozoa (Acridine Orange test)</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>4.5.6.1</td>
<td>DNA damaged of spermatozoa of spermatozoa at (Acridine Orange test) pre-freezing (5°C) stage</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td>4.5.6.2</td>
<td>DNA damaged of spermatozoa of spermatozoa at (Acridine Orange test) post-freezing (24 hours) stage</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td>4.5.6.3</td>
<td>DNA damaged of spermatozoa of spermatozoa at (Acridine Orange test) post-freezing (72 hours) stage</td>
<td>132</td>
<td></td>
</tr>
<tr>
<td>4.5.6.4</td>
<td>DNA damaged of spermatozoa of spermatozoa at (Acridine Orange test) in TYG dilutor</td>
<td>133</td>
<td></td>
</tr>
<tr>
<td>4.5.6.5</td>
<td>DNA damaged of spermatozoa of spermatozoa at (Acridine Orange test) in TYD dilutor</td>
<td>133</td>
<td></td>
</tr>
<tr>
<td>4.5.6.6</td>
<td>DNA damaged of spermatozoa of spermatozoa at (Acridine Orange test) in TYE dilutor</td>
<td>134</td>
<td></td>
</tr>
<tr>
<td>4.5.7</td>
<td>DNA damaged spermatozoa percentage (Comet assay)</td>
<td>136</td>
<td></td>
</tr>
<tr>
<td>4.5.7.1</td>
<td>DNA damaged spermatozoa percentage (Comet assay) at pre-freezing (5°C) stage</td>
<td>137</td>
<td></td>
</tr>
<tr>
<td>4.5.7.2</td>
<td>DNA damaged spermatozoa percentage (Comet assay) at post-freezing (24 hours) stage</td>
<td>137</td>
<td></td>
</tr>
<tr>
<td>4.5.7.3</td>
<td>DNA damaged spermatozoa percentage</td>
<td>138</td>
<td></td>
</tr>
<tr>
<td>Section</td>
<td>Description</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4.5.7.4</td>
<td>DNA damaged spermatozoa percentage (Comet assay) at post-freezing (72 hours) stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5.7.5</td>
<td>DNA damaged spermatozoa percentage (Comet assay) in TYG dilutor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5.7.6</td>
<td>DNA damaged spermatozoa percentage (Comet assay) in TYE dilutor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5.8</td>
<td>Glutamic–Oxaloacetic Transaminase (GOT) and Glutamic–Pyruvate Transaminase (GPT) activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5.8.1</td>
<td>GOT and GPT at pre-freezing (5°C) stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5.8.2</td>
<td>GOT and GPT at post-freezing (24 hours) stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5.8.3</td>
<td>GOT and GPT at post-freezing (72 hours) stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5.8.4</td>
<td>GOT and GPT in TYG dilutor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5.8.5</td>
<td>GOT and GPT in TYD dilutor</td>
<td></td>
<td></td>
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<tr>
<td>4.5.8.6</td>
<td>GOT and GPT in TYE dilutor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5.9</td>
<td>Alkaline phosphatase (AKP) and Acid phosphatase activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5.9.1</td>
<td>Alkaline phosphatase (AKP) and Acid phosphatase activity at pre-freezing (5°C) stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5.9.2</td>
<td>Alkaline phosphatase (AKP) and Acid phosphatase activity at post-freezing (24 hours) stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5.9.3</td>
<td>Alkaline phosphatase (AKP) and Acid phosphatase activity at post-freezing (72 hours) stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5.9.4</td>
<td>Alkaline phosphatase (AKP) and Acid phosphatase activity in TYG dilutor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5.9.5</td>
<td>Alkaline phosphatase (AKP) and Acid phosphatase activity in TYD dilutor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5.9.6</td>
<td>Alkaline phosphatase (AKP) and Acid phosphatase activity in TYE dilutor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5.10</td>
<td>Lactate dehydrogenase (LDH) activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5.10.1</td>
<td>Lactate dehydrogenase (LDH) activity at pre-freezing (5°C) stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5.10.2</td>
<td>Lactate dehydrogenase (LDH) activity at post-freezing (24 hours) stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5.10.3</td>
<td>Lactate dehydrogenase (LDH) activity at post-freezing (72 hours) stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5.10.4</td>
<td>Lactate dehydrogenase (LDH) activity in TYG dilutor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5.10.5</td>
<td>Lactate dehydrogenase (LDH) activity in TYD dilutor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5.10.6</td>
<td>Lactate dehydrogenase (LDH) activity in TYE dilutor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.6</td>
<td>Study of conception rate of frozen semen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.6.1</td>
<td>Conception rate of Osmanabadi buck frozen semen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Section</td>
<td>Description</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>4.6.2</td>
<td>Conception rate of Sirohi buck frozen semen</td>
<td>166</td>
<td></td>
</tr>
<tr>
<td>4.6.3</td>
<td>Overall conception rate of Osmanabadi and Sirohi buck frozen semen in different dilutors</td>
<td>167</td>
<td></td>
</tr>
<tr>
<td>4.7</td>
<td>Evaluation of Boer and Damascus bucks pelleted frozen semen received from research organization</td>
<td>167</td>
<td></td>
</tr>
<tr>
<td>4.7.1</td>
<td>Evaluation of semen quality of pellets of Boer and Damascus breed of bucks</td>
<td>167</td>
<td></td>
</tr>
<tr>
<td>4.7.2</td>
<td>Conception rate of Boer and Damascus bucks pelleted frozen semen</td>
<td>169</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Summary and Conclusion</td>
<td>170-226</td>
<td></td>
</tr>
<tr>
<td>5.1</td>
<td>Macroscopic evaluation of semen of Osmanabadi and Sirohi bucks</td>
<td>171</td>
<td></td>
</tr>
<tr>
<td>5.1.1</td>
<td>Ejaculate volume</td>
<td>171</td>
<td></td>
</tr>
<tr>
<td>5.1.2</td>
<td>Colour, Consistency and Density of neat semen.</td>
<td>171</td>
<td></td>
</tr>
<tr>
<td>5.1.3</td>
<td>Hydrogen ion concentration (pH) of semen</td>
<td>171</td>
<td></td>
</tr>
<tr>
<td>5.1.4</td>
<td>Resazurin test</td>
<td>171</td>
<td></td>
</tr>
<tr>
<td>5.2</td>
<td>Microscopic evaluation of semen of Osmanabadi and Sirohi bucks</td>
<td>172</td>
<td></td>
</tr>
<tr>
<td>5.2.1</td>
<td>Mass activity</td>
<td>172</td>
<td></td>
</tr>
<tr>
<td>5.2.2</td>
<td>Total sperm concentration</td>
<td>172</td>
<td></td>
</tr>
<tr>
<td>5.2.3</td>
<td>Initial sperm motility percent in neat semen.</td>
<td>172</td>
<td></td>
</tr>
<tr>
<td>5.2.4</td>
<td>Live sperm percentage in neat semen</td>
<td>173</td>
<td></td>
</tr>
<tr>
<td>5.2.5</td>
<td>Abnormal sperm percentage in neat semen.</td>
<td>173</td>
<td></td>
</tr>
<tr>
<td>5.3</td>
<td>Evaluation of neat semen of Osmanabadi and Sirohi bucks using sperm function tests</td>
<td>173</td>
<td></td>
</tr>
<tr>
<td>5.3.1</td>
<td>Plasma membrane integrity (Hypo-Osmotic Swelling Test- HOST)</td>
<td>173</td>
<td></td>
</tr>
<tr>
<td>5.3.2</td>
<td>Acrosome integrity (Giemsa stain)</td>
<td>174</td>
<td></td>
</tr>
<tr>
<td>5.3.3</td>
<td>DNA damage</td>
<td>174</td>
<td></td>
</tr>
<tr>
<td>5.3.3.1</td>
<td>DNA damage (Acridine orange test)</td>
<td>174</td>
<td></td>
</tr>
<tr>
<td>5.3.3.2</td>
<td>DNA damage (single gel electrophoresis-Comet assay)</td>
<td>174</td>
<td></td>
</tr>
<tr>
<td>5.4</td>
<td>Enzymes in seminal plasma of Osmanabadi and Sirohi bucks</td>
<td>174</td>
<td></td>
</tr>
<tr>
<td>5.4.1</td>
<td>Transaminase (GOT and GPT)</td>
<td>174</td>
<td></td>
</tr>
<tr>
<td>5.4.2</td>
<td>Alkaline phosphatase (AKP) and Acid phosphatase (ACP)</td>
<td>175</td>
<td></td>
</tr>
<tr>
<td>5.4.3</td>
<td>Lactate Dehydrogenase (LDH)</td>
<td>175</td>
<td></td>
</tr>
<tr>
<td>5.5</td>
<td>Evaluation of semen quality of Osmanabadi and Sirohi bucks at different freezing stages in different dilutors</td>
<td>176</td>
<td></td>
</tr>
<tr>
<td>5.5.1</td>
<td>Sperm Motility</td>
<td>176</td>
<td></td>
</tr>
<tr>
<td>5.5.1.1</td>
<td>Sperm motility percentage at pre-freezing (5OC) stage</td>
<td>176</td>
<td></td>
</tr>
<tr>
<td>5.5.1.2</td>
<td>Sperm motility percentage at post-freezing (24 hours) stage</td>
<td>177</td>
<td></td>
</tr>
<tr>
<td>5.5.1.3</td>
<td>Sperm motility percentage at post-freezing (72 hours) stage</td>
<td>177</td>
<td></td>
</tr>
<tr>
<td>5.5.1.4</td>
<td>Sperm motility percentage in TYG dilutor</td>
<td>178</td>
<td></td>
</tr>
<tr>
<td>5.5.1.5</td>
<td>Sperm motility percentage in TYE dilutor</td>
<td>179</td>
<td></td>
</tr>
<tr>
<td>5.5.1.6</td>
<td>Sperm motility percentage in TYD dilutor</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>5.5.2</td>
<td>Live sperm percentage</td>
<td>181</td>
<td></td>
</tr>
<tr>
<td>5.5.2.1</td>
<td>Live sperm percentage at pre-freezing (5°C) stage</td>
<td>181</td>
<td></td>
</tr>
<tr>
<td>5.5.2.2</td>
<td>Live sperm percentage at post-freezing (24 hours) stage</td>
<td>181</td>
<td></td>
</tr>
<tr>
<td>5.5.2.3</td>
<td>Live sperm percentage at post-freezing (72 hours) stage</td>
<td>182</td>
<td></td>
</tr>
<tr>
<td>5.5.2.4</td>
<td>Live sperm percentage TYG dilutor</td>
<td>182</td>
<td></td>
</tr>
<tr>
<td>5.5.2.5</td>
<td>Live sperm percentage TYD dilutor</td>
<td>183</td>
<td></td>
</tr>
<tr>
<td>5.5.2.6</td>
<td>Live sperm percentage TYE dilutor</td>
<td>184</td>
<td></td>
</tr>
<tr>
<td>5.5.3</td>
<td>Abnormal sperm percentage</td>
<td>185</td>
<td></td>
</tr>
<tr>
<td>5.5.3.1</td>
<td>Abnormal sperm percentage at pre-freezing (5°C) stage</td>
<td>185</td>
<td></td>
</tr>
<tr>
<td>5.5.3.2</td>
<td>Abnormal sperm percentage at post-freezing (24 hours) stage</td>
<td>185</td>
<td></td>
</tr>
<tr>
<td>5.5.3.3</td>
<td>Abnormal sperm percentage at post-freezing (72 hours) stage</td>
<td>186</td>
<td></td>
</tr>
<tr>
<td>5.5.3.4</td>
<td>Abnormal sperm percentage in TYG dilutor</td>
<td>187</td>
<td></td>
</tr>
<tr>
<td>5.5.3.5</td>
<td>Abnormal sperm percentage in TYE dilutor</td>
<td>188</td>
<td></td>
</tr>
<tr>
<td>5.5.3.6</td>
<td>Abnormal sperm percentage in TYD dilutor</td>
<td>189</td>
<td></td>
</tr>
<tr>
<td>5.5.4</td>
<td>Plasma membrane integrity of spermatozoa (HOST)</td>
<td>190</td>
<td></td>
</tr>
<tr>
<td>5.5.4.1</td>
<td>Plasma membrane integrity of spermatozoa at pre-freezing (50°C) stage</td>
<td>190</td>
<td></td>
</tr>
<tr>
<td>5.5.4.2</td>
<td>Plasma membrane integrity of spermatozoa at post-freezing (24 hours) stage</td>
<td>190</td>
<td></td>
</tr>
<tr>
<td>5.5.4.3</td>
<td>Plasma membrane integrity of spermatozoa at post-freezing (72 hours) stage</td>
<td>191</td>
<td></td>
</tr>
<tr>
<td>5.5.4.4</td>
<td>Plasma membrane integrity of spermatozoa in TYG dilutor</td>
<td>192</td>
<td></td>
</tr>
<tr>
<td>5.5.4.5</td>
<td>Plasma membrane integrity of spermatozoa in TYD dilutor</td>
<td>193</td>
<td></td>
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<tr>
<td>5.5.4.6</td>
<td>Plasma membrane integrity of spermatozoa in TYE dilutor</td>
<td>193</td>
<td></td>
</tr>
<tr>
<td>5.5.5</td>
<td>Acrosome intactness of spermatozoa (Giemsa stain)</td>
<td>194</td>
<td></td>
</tr>
<tr>
<td>5.5.5.1</td>
<td>Acrosome intactness of spermatozoa at pre-freezing (5°C) stage</td>
<td>195</td>
<td></td>
</tr>
<tr>
<td>5.5.5.2</td>
<td>Acrosome intactness of spermatozoa at post-freezing (24 hours) stage</td>
<td>195</td>
<td></td>
</tr>
<tr>
<td>5.5.5.3</td>
<td>Acrosome intactness of spermatozoa at post-freezing (72 hours) stage</td>
<td>196</td>
<td></td>
</tr>
<tr>
<td>5.5.5.4</td>
<td>Acrosome intactness of spermatozoa in TYG dilutor</td>
<td>196</td>
<td></td>
</tr>
<tr>
<td>5.5.5.5</td>
<td>Acrosome intactness of spermatozoa in TYD dilutor</td>
<td>197</td>
<td></td>
</tr>
<tr>
<td>Section</td>
<td>Description</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>5.5.6</td>
<td>Acrosome intactness of spermatozoa in TYE dilutor</td>
<td>198</td>
<td></td>
</tr>
<tr>
<td>5.5.6.1</td>
<td>DNA damaged of spermatozoa (Acridine Orange test)</td>
<td>199</td>
<td></td>
</tr>
<tr>
<td>5.5.6.2</td>
<td>DNA damaged of spermatozoa of spermatozoa at (Acridine Orange test) pre-freezing (5°C) stage</td>
<td>199</td>
<td></td>
</tr>
<tr>
<td>5.5.6.3</td>
<td>DNA damaged of spermatozoa of spermatozoa at (Acridine Orange test) at post-freezing (24 hours) stage</td>
<td>199</td>
<td></td>
</tr>
<tr>
<td>5.5.6.4</td>
<td>DNA damaged of spermatozoa of spermatozoa at (Acridine Orange test) at post-freezing (72 hours) stage</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>5.5.6.5</td>
<td>DNA damaged of spermatozoa of spermatozoa at (Acridine Orange test) in TYG dilutor</td>
<td>201</td>
<td></td>
</tr>
<tr>
<td>5.5.6.6</td>
<td>DNA damaged of spermatozoa of spermatozoa at (Acridine Orange test) in TYD dilutor</td>
<td>201</td>
<td></td>
</tr>
<tr>
<td>5.5.7</td>
<td>DNA damaged of spermatozoa of spermatozoa at (Acridine Orange test) in TYE dilutor</td>
<td>202</td>
<td></td>
</tr>
<tr>
<td>5.5.7.1</td>
<td>DNA damaged spermatozoa percentage (Comet assay)</td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>5.5.7.2</td>
<td>DNA damaged spermatozoa percentage (Comet assay) at pre-freezing (5°C) stage</td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>5.5.7.3</td>
<td>DNA damaged spermatozoa percentage (Comet assay) at post-freezing (24 hours) stage</td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>5.5.7.4</td>
<td>DNA damaged spermatozoa percentage (Comet assay) in TYG dilutor</td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>5.5.7.5</td>
<td>DNA damaged spermatozoa percentage (Comet assay) in TYD dilutor</td>
<td>204</td>
<td></td>
</tr>
<tr>
<td>5.5.7.6</td>
<td>DNA damaged spermatozoa percentage (Comet assay) in TYE dilutor</td>
<td>204</td>
<td></td>
</tr>
<tr>
<td>5.5.8</td>
<td>Glutamic – Oxaloacetic Transaminase (GOT) and Glutamic – Pyruvate Transaminase (GPT) activity</td>
<td>205</td>
<td></td>
</tr>
<tr>
<td>5.5.8.1</td>
<td>GOT and GPT at pre-freezing (5°C) stage.</td>
<td>206</td>
<td></td>
</tr>
<tr>
<td>5.5.8.2</td>
<td>GOT and GPT at post-freezing (24 hours) stage</td>
<td>206</td>
<td></td>
</tr>
<tr>
<td>5.5.8.3</td>
<td>GOT and GPT at post-freezing (72 hours) stage</td>
<td>207</td>
<td></td>
</tr>
<tr>
<td>5.5.8.4</td>
<td>GOT and GPT in TYG dilutor</td>
<td>208</td>
<td></td>
</tr>
<tr>
<td>5.5.8.5</td>
<td>GOT and GPT in TYD dilutor</td>
<td>209</td>
<td></td>
</tr>
<tr>
<td>5.5.8.6</td>
<td>GOT and GPT in TYE dilutor</td>
<td>210</td>
<td></td>
</tr>
<tr>
<td>5.5.9</td>
<td>Alkaline phosphatase (AKP) and Acid phosphatase activity</td>
<td>212</td>
<td></td>
</tr>
<tr>
<td>5.5.9.1</td>
<td>Alkaline phosphatase (AKP) and Acid phosphatase activity at pre-freezing (5°C) stage.</td>
<td>213</td>
<td></td>
</tr>
<tr>
<td>5.5.9.2</td>
<td>Alkaline phosphatase (AKP) and Acid phosphatase activity at post-freezing (24 hours) stage</td>
<td>214</td>
<td></td>
</tr>
<tr>
<td>5.5.9.3</td>
<td>Alkaline phosphatase (AKP) and Acid phosphatase activity</td>
<td>215</td>
<td></td>
</tr>
<tr>
<td>Section</td>
<td>Description</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>5.5.9.4</td>
<td>Alkaline phosphatase (AKP) and Acid phosphatase activity in TYG dilutor</td>
<td>216</td>
<td></td>
</tr>
<tr>
<td>5.5.9.5</td>
<td>Alkaline phosphatase (AKP) and Acid phosphatase activity in TYD dilutor</td>
<td>217</td>
<td></td>
</tr>
<tr>
<td>5.5.9.6</td>
<td>Alkaline phosphatase (AKP) and Acid phosphatase activity in TYE dilutor</td>
<td>218</td>
<td></td>
</tr>
<tr>
<td>5.5.10</td>
<td>Lactate dehydrogenase (LDH) activity</td>
<td>219</td>
<td></td>
</tr>
<tr>
<td>5.5.10.1</td>
<td>Lactate dehydrogenase (LDH) activity at pre-freezing (5°C) stage.</td>
<td>219</td>
<td></td>
</tr>
<tr>
<td>5.5.10.2</td>
<td>Lactate dehydrogenase (LDH) activity at post-freezing (24 hours) stage</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>5.5.10.3</td>
<td>Lactate dehydrogenase (LDH) activity at post-freezing (72 hours) stage</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>5.5.10.4</td>
<td>Lactate dehydrogenase (LDH) activity in TYG dilutor</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>5.5.10.5</td>
<td>Lactate dehydrogenase (LDH) activity in TYD dilutor</td>
<td>221</td>
<td></td>
</tr>
<tr>
<td>5.5.10.6</td>
<td>Lactate dehydrogenase (LDH) activity in TYE dilutor</td>
<td>221</td>
<td></td>
</tr>
<tr>
<td>5.6</td>
<td>Study of conception rate of frozen semen</td>
<td>222</td>
<td></td>
</tr>
<tr>
<td>5.6.1</td>
<td>Conception rate of Osmanabadi buck frozen semen</td>
<td>222</td>
<td></td>
</tr>
<tr>
<td>5.6.2</td>
<td>Conception rate of Sirohi buck frozen semen</td>
<td>222</td>
<td></td>
</tr>
<tr>
<td>5.6.3</td>
<td>Overall conception rate of Osmanabadi and Sirohi buck frozen semen in different dilutors</td>
<td>223</td>
<td></td>
</tr>
<tr>
<td>5.7</td>
<td>Evaluation of semen quality of pellets of Boer and Damascus breed of bucks</td>
<td>223</td>
<td></td>
</tr>
<tr>
<td>5.7.1</td>
<td>Conception rate of Boer and Damascus bucks pelleted frozen semen</td>
<td>224</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>PROPOSED AREA FOR FUTURE RESEARCH WORK</td>
<td>227</td>
<td></td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table Number</th>
<th>Name of table</th>
<th>Page number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Seminal attributes of neat semen of Osmanabadi and Sirohi bucks</td>
<td>73</td>
</tr>
<tr>
<td>2.</td>
<td>Time (minutes) required for changing colour in resazurin reduction test</td>
<td>76</td>
</tr>
<tr>
<td>3.</td>
<td>Sperm motility percentage at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks</td>
<td>90</td>
</tr>
<tr>
<td>4.</td>
<td>Live sperm percentage at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks</td>
<td>99</td>
</tr>
<tr>
<td>5.</td>
<td>Abnormal sperm percentage at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks</td>
<td>106</td>
</tr>
<tr>
<td>6.</td>
<td>Plasma membrane integrity percentage of spermatozoa at different freezing stages in different dilutors of Osmanabadi and Sirohi bucks (HOST)</td>
<td>114</td>
</tr>
<tr>
<td>7.</td>
<td>Acrosome intact sperm percentage at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks (Giemsa stain)</td>
<td>122</td>
</tr>
<tr>
<td>8.</td>
<td>DNA damaged of sperm percentage at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks (Acridine Orange test)</td>
<td>129</td>
</tr>
<tr>
<td>9.</td>
<td>DNA damaged spermatozoa percentage at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks (Comet assay)</td>
<td>135</td>
</tr>
<tr>
<td>10.</td>
<td>Transaminase (GOT and GPTIU/L) activity at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks semen</td>
<td>140</td>
</tr>
<tr>
<td>11.</td>
<td>Alkaline phosphatase (AKP) at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks</td>
<td>149</td>
</tr>
<tr>
<td>12.</td>
<td>Lactate dehydrogenase ((LDH) at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks</td>
<td>158</td>
</tr>
<tr>
<td>13.</td>
<td>Conception rate of Osmanabadi buck frozen semen in different dilutors</td>
<td>163</td>
</tr>
<tr>
<td>14.</td>
<td>Conception rate of Sirohi buck frozen semen in different dilutors</td>
<td>164</td>
</tr>
<tr>
<td>15.</td>
<td>Over all conception rate of Osmanabadi and Sirohi buck frozen semen in different dilutors</td>
<td>165</td>
</tr>
<tr>
<td>16.</td>
<td>Quality of Boer and Damascus bucks pelleted frozen semen</td>
<td>167</td>
</tr>
<tr>
<td>17.</td>
<td>Conception rate of Boer and Damascus buck pelleted frozen semen</td>
<td>168</td>
</tr>
</tbody>
</table>
## List of figures

<table>
<thead>
<tr>
<th>Figure. No</th>
<th>Title of figure</th>
<th>Between Page No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Selected bucks</td>
<td>55-56</td>
</tr>
<tr>
<td>2</td>
<td>Semen collection from selected bucks</td>
<td>57-58</td>
</tr>
<tr>
<td>3</td>
<td>Semen preservation</td>
<td>68-69</td>
</tr>
<tr>
<td>4</td>
<td>Oestrous synchronization and Artificial Insemination in does.</td>
<td>71-72</td>
</tr>
<tr>
<td>5</td>
<td>Semen ejaculate</td>
<td>75-76</td>
</tr>
<tr>
<td>6</td>
<td>Resazurin test</td>
<td>77-78</td>
</tr>
<tr>
<td>7</td>
<td>Sperm Acrosome Intactness test of neat semen (Giemsa stain)</td>
<td>85-86</td>
</tr>
<tr>
<td>8</td>
<td>Sperm Acrosome Intactness test of thawed semen (Giemsa stain)</td>
<td>117-118</td>
</tr>
<tr>
<td>9</td>
<td>Hypo-Osmotic Swelling Test of post thaw semen (HOST)</td>
<td>125-126</td>
</tr>
<tr>
<td>10</td>
<td>Acridin orange test for DNA damage in neat semen</td>
<td>130-131</td>
</tr>
<tr>
<td>11</td>
<td>Acridin orange test for DNA damage in post thaw semen.</td>
<td>130-131</td>
</tr>
<tr>
<td>12</td>
<td>DNA damage test for post thaw semen (Comet assay)</td>
<td>137-138</td>
</tr>
<tr>
<td>13</td>
<td>Osmanabadi X Sirohi kids born through AI</td>
<td>165-166</td>
</tr>
<tr>
<td>14</td>
<td>Osmanabadi X Boer kids born through AI</td>
<td>165-166</td>
</tr>
<tr>
<td>15</td>
<td>Osmanabadi X Damascus kids born through AI</td>
<td>165-166</td>
</tr>
</tbody>
</table>
ABBREVIATIONS

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>µmol/L</td>
<td>Micro mol per liter</td>
</tr>
<tr>
<td>A.V</td>
<td>Artificial Vagina</td>
</tr>
<tr>
<td>ACP</td>
<td>Acid Phosphatase</td>
</tr>
<tr>
<td>AI</td>
<td>Artificial Insemination</td>
</tr>
<tr>
<td>AKP</td>
<td>Alkaline Phosphatase</td>
</tr>
<tr>
<td>AO</td>
<td>Acridin Orange</td>
</tr>
<tr>
<td>AOT</td>
<td>Acridin Orange Test</td>
</tr>
<tr>
<td>BU/100ml</td>
<td>Bodansky Unit per 100ml</td>
</tr>
<tr>
<td>C.B.</td>
<td>Crossbred</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>C.C.</td>
<td>Cubic Centimeter</td>
</tr>
<tr>
<td>DCKL</td>
<td>Development Corporation Konkan Limited</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>E.N.</td>
<td>Eosin- Nigrosin</td>
</tr>
<tr>
<td>EG</td>
<td>Ethylene glycol</td>
</tr>
<tr>
<td>EYC</td>
<td>Egg Yolk Citrate</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>GOT</td>
<td>Glutamic Oxalo-acetic Transaminase</td>
</tr>
<tr>
<td>GPT</td>
<td>Glutamic Pyruvic Transaminase</td>
</tr>
<tr>
<td>HF</td>
<td>Holstein Friesian</td>
</tr>
<tr>
<td>HOS test</td>
<td>Hypo Osmotic Swelling Test</td>
</tr>
<tr>
<td>hr</td>
<td>Hour</td>
</tr>
<tr>
<td>IU/L</td>
<td>International Unit per liter</td>
</tr>
<tr>
<td>KAU/100ml</td>
<td>King and Armstrong Unit</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactic dehydrogenase</td>
</tr>
<tr>
<td>LN</td>
<td>Liquid Nitrogen</td>
</tr>
<tr>
<td>NIRRH</td>
<td>National Institute for Research in Reproductive Health</td>
</tr>
<tr>
<td>min.</td>
<td>Minutes</td>
</tr>
<tr>
<td>ml</td>
<td>Milliliter</td>
</tr>
<tr>
<td>PMI</td>
<td>Plasma Membrane Integrity</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>RRT</td>
<td>Resazurin Reduction Test</td>
</tr>
<tr>
<td>SCSA</td>
<td>Sperm Chromatin Structure Assay</td>
</tr>
<tr>
<td>S.E.</td>
<td>Standard error</td>
</tr>
<tr>
<td>TYG</td>
<td>Tris Egg yolk Glycerol</td>
</tr>
<tr>
<td>TYD</td>
<td>Tris Egg yolk Di-methyl Sulphoxide</td>
</tr>
<tr>
<td>TYE</td>
<td>Tris egg yolk Ethylene glycol</td>
</tr>
<tr>
<td>U/L</td>
<td>International unit per liter</td>
</tr>
</tbody>
</table>

1. INTRODUCTION
Goats were one of the earliest animals to be domesticated and are underated as Farm Livestock. The goat meat is lean, has low fat, and is liked by all. Goat meat is the primary source of protein in many parts of the world. Worldwide, goat's milk is consumed more than cow's milk. Goats are also an important source of fiber and skins. In many areas, there is a growing interest in raising goats for meat. The demand of goat meat is increasing, even though the price is high. Their population in the country is continuously increasing, though 60% of the goats are slaughtered every year. There has been a renewed interest in contribution of goats in meeting the world's needs for food. The resources available and their location should be considered in any attempt to maximize contributions of this species. In many countries, goats are mainly raised for meat production. Goats are important Livestock species in developing countries. Of the world's 475 million goats, 95 percent are located in developing countries. 60% of the world's goat population resides in Asia (FAO 2003), India accounts for 20% of this with the annual growth rate of 1.6% in spite of 38% annual slaughter rate and approximately 15% mortality (Department of Animal Husbandry and Dairying 2005). Overall India has 9.8% (182 million) of the total small ruminants of the world, comprising of 61 million sheep and 121 million of goat population. Contribution of goat has an overwhelming impact when it comes to the rural India. They are usually associated with the poorest of the poor and many a time the goat has been the only source of income for a family. That is why; it has rightly been called "Poor Man's Cow" by Mahatma Ganghi. Goat is a multi functional animal and plays a significant role in the economy and nutrition of landless, small and marginal Farmers in the country. In spite of the fact that the goat is of paramount importance to smallholders and to the local meat markets, only a few isolated attempts at improving its production through controlled management have been made. In addition, little attention has been paid to the indigenous tropical breeds.

In South Asia, small ruminants contribute enormously towards promotion of livelihoods security and as an insurance cover to cope with crop failures particularly for rural landless, small and marginal Farmers (Pasha 2000, Misra 2005). Goat Farming is also increasingly being taken up by peri-urban poor population due to easy market access and as a source of nutritional security for the household (Pollot and Wilson 2009). Worldwide upward trends in goat numbers are shown but progress is at a rate of only about one percent per year. Goat numbers have increased from 0.4
billion to 0.5 billion in about 20 years. Further increases are not only likely but probable. The rate of increase in numbers could be much greater. Numbers have increased more rapidly in developing than in developed countries although developed countries are now showing increases.

There is a paucity of scientific information on goat reproduction to help improve goat herd management. Reproduction and genetics of the goat are reviewed with a view of increasing their contribution to mankind. Excellent genotypes for producing milk and fiber are available, but adaptation to tropical conditions is needed. Little has been done on the development of the goat as a meat animal. Also, research on crossbreeding for milk or meat production is limited.

The productivity of Indian goats is low, yet considering the nutritional and physical environmental conditions under which they are reared, it cannot be considered inefficient. The main lacuna in case of goats is the non availability of improved breeds which could be utilized. As these animals are owned by the poor strata of society and there is a mistaken prejudice that they are responsible for environmental degradation; no sustained efforts have been made to improve indigenous breeds. Indigenous breeds have developed over the centuries to survive and adapt themselves to harsh environmental conditions but do not universally possess ability to respond to better management and nutrition.

Major reasons for this low productivity are inadequate grazing resources, disease problems and serious lack of organized efforts for genetic improvement. Among the Indian breeds, Osmanabadi goat shows a very efficient reproductive performance and resistance against diseases not only in well managed semi stall feeding system but also in severe drought conditions. As per research conducted on Osmanabadi Goats in Maharashtra, in spite of drought which occurred in 2007 and 2008, there was no drop in the productivity rate of Osmanabadi goat. The age at first kidding in Osmanabadi goat is around 15 months with the lactation length of around 130 days. The dressing percentage varies from 55-60 %. In favourable conditions the does will breed twice a year, twinning is common and the daily milk yield ranged from 700 to 1500 g. Beside Osmanabadi goats, Sirohi is also one of the Indigenous breed which also thrives well in arid regions and also known for its high milk yield and meat quality. On an average, the birth weight is about 2.0 kg. The age at first kidding is 19-20 months and the litter size is one kid per birth. This breed also shows good growth rate in kids. The breed is well suited to stall feeding. Hence cross breeding of
Osmanabadi and Sirohi may show improved growth rate in kids with optimum weight of kids at salable age should be about 28 to 30 kg.

Buck is very critical to the success of breeding program, which contributes almost half of all the genetic material of every kid. But little importance is given for selection of bucks used for breeding, and much inter-mating among neighboring breeds takes place. This is why it is critical to select good quality bucks that will move herd in a positive direction. Bucks should be selected well before the start of the breeding season. Selection of buck can be done on the basis of semen evaluation. Semen analysis is a valuable diagnostic tool to assess the fertility status of the male. Semen quality testing is critical because each male is frequently bred to many females. Routine techniques in semen examination for fertility are only related to the number and motility (movement) of sperm. Infertility is discovered only when semen is used and females do not become pregnant. Non-pregnant does are at risk of long inter-kidding period, reduced fertility, resulting in economic losses and loss of production. However, the prediction of potential fertility of a male on the basis of a single seminal assay is not reliable. Conventional parameters used for evaluation of semen have limited application, because they only help to assess the structural integrity of the cell (Neild et al., 1999). Each sperm cell consists of multiple sub cellular compartments with different functions, all of which must be intact for successful fertilization (Amann and Graham, 1993). The major draw back in routine semen analysis is that it has limited prognostic value because it cannot evaluate the functional competence of semen to fertilize ovum. Conventional semen analysis therefore provides information about sperm count, motility and morphology of sperm; however, it fails to provide information about the ability of sperm to fertilize oocyte. Hence, this necessitates studying the sperm function test to know the ability of sperm to fertilize the oocyte before the semen sample can be utilized for its preservation.

An intact plasma membrane is required for normal sperm functions. In recent years, more attention has been given for evaluating sperm membrane integrity as it is of fundamental importance in the fertilization process. Membrane integrity is not only important for sperm metabolism, but a correct change in the properties of the membrane is required for successful union of the male and female gametes, i.e. for sperm capacitation, acrosome reaction, and binding of spermatozoon to the egg surface. Thus, the integrity and functional activity of the sperm membrane is of
fundamental importance in the fertilization process, and assessment of membrane function may be a useful indicator of the fertilizing ability of spermatozoa.

Fertilization also involves processes that are intrinsically related to the sperm cell membrane integrity. Any trauma to membrane like destruction by ice crystals formation during freezing may prevent sperm function normally. The extracellular release of GOT and GPT enzymes have been used as test enzymes and thereby indicators of sperm cell membrane damage after freezing. Transaminase has an important role in catabolism of glutamate by bovine spermatozoa.

The processes of cooling, freezing and thawing generate physical and chemical stresses on the sperm membranes, thereby reducing sperm viability and fertilizing ability. One of the deleterious effects of cryopreservation is the generation of reactive oxygen species (ROS) (Alvarez and Storey, 1992; Chatterjee and Gagnon, 2001). Moreover, freezing and thawing of spermatozoa increase the generation of ROS resulting in DNA damage (Lopes et al., 1998). DNA damage can have a significant negative impact on oocyte fertilization, embryo development, and live-birth rate. One way to overcome the detrimental effects of ROS on sperm performance after thawing may be addition of different cryopreservatives (Glycerol/Ethylene Glycol/DMSO) to the freezing extender.

Generally, there is no difficulty in collecting freezable quality semen from mature, experienced bucks. But not all fertile males produce semen that freezes well, and of those that do, not all samples collected will freeze well. The goal in semen freezing is to produce insemination doses that contain a minimum number of good quality sperm cells (an insemination dose is the amount of frozen semen required for one insemination and is packaged in individual straws). Males that produce large amounts of excellent quality semen will require fewer collections to produce the insemination doses ordered. Greater the number of good sperm cells in a dose, the better the fertility. For good fertility adjusts samples with an insemination dose with a minimum of 50 million good quality sperm - typically 100 million total sperm per dose. Satisfactory conception rates have been achieved using washed or unwashed frozen-thawed semen extended in egg yolk diluents. It seems that the best goat sperm extender is Tris- citric acid, fructose, egg yolk, glycerol. Purohit et al. (1992) did not find any difference between direct dilution and washing of sperm before dilution with this extender. Research found out that 5% glycerol is superior to 3 to 4% glycerol in extenders. Singh et al. (1995) recommended similar extender containing 6% glycerol
and 1% dimethyl sulfoxide (DMSO) and also, a combination of glycerol with lactose is a better cryoprotectant than glycerol alone.

Artificial Insemination is the tool to improve the genetic potential in animals. AI has many benefits for producers. However, it is important that producers have realistic expectations from the results of AI programs. Many have expectations that are well beyond what is normally achieved. A good benchmark is to compare the results of small ruminant AI against the results of cattle AI programs - which have had many millions of dollars investing in improvements over 25 years. The average number of dairy cattle that get pregnant from a single insemination using frozen semen is reported from around the world to be about 50%. AI in small ruminants is more difficult than in cattle because of the small animal size and the Complex anatomy of the cervical canal - making insemination into the uterus difficult. Sheep are the most difficult, goats are the easiest and deer are between the two in difficulty. The process of freezing and thawing semen damages many of sperm cells which causes reduced fertility. Results are affected by the numbers of sperm that survived the freezing and thawing. Frozen semen rarely produces fertility as good as fresh semen. To get best pregnancy rates, frozen semen must be deposited into the uterus. Cervical AI is used to introduce semen into the cervical canal. Cervical AI is used commonly in goats, particularly because many types of insemination equipment are not designed for passage through the cervical canal into the uterus. Results are considerably better when semen is deposited into uterus than depositing in vagina.

Fertility trials are the ultimate tests to evaluate reproductive performance, these trials only can assure about the quality of semen used in AI procedure.

With consideration of all above facts in mind the present study was designed with following objectives.

1. To study the quality of neat semen of Osmanabadi and Sirohi bucks by using macroscopic, microscopic and sperm function tests

2. To compare pre-freeze quality and post freezing quality of Osmanabadi and Sirohi buck semen in three different dilutors using, microscopic, sperm function and enzyme leakage tests
3. To evaluate the effect of dilutors on quality of Osmanabadi and Sirohi buck semen at post freeze stage (at 24 hrs & 72 hrs) by using various microscopic, sperm function tests and enzyme leakage tests

4. To study post thaw quality of frozen semen pellets of Boer and Damascus breed bucks purchased from a research organization by using various microscopic, sperm function tests and enzyme leakage tests

5. To study conception rate in goats inseminated using frozen semen of Osmanabadi and Sirohi buck

6. To study conception rate in goats inseminated using frozen semen of Boer and Damascus buck purchased from research organization.
2. REVIEW OF LITERATURE

The present study was carried out on “Deep-Freezing of Osmanabadi and Sirohi buck semen with special reference to sperm membrane integrity, DNA damage and fertilizing ability” in three different dilutors. The relevant scientific literature on these aspects has been reviewed under the following sub-heads.

2.1 Macroscopic evaluation of semen.
2.2 Microscopic evaluation of semen
2.3 Sperm function tests
2.4 Enzyme leakage
2.5 Conception rates with frozen goat semen used for Artificial Insemination.

2.1 Macroscopic evaluation of semen

All seminal attributes play a major role in deciding the quality of semen as well as its future fertility. Visual appraisal of ejaculate volume, colour, consistency and density have continued to be simple and rapid semen evaluation tests, which are routinely used by AI technician/ semen bank/ AI centre. These are traditional methods which give rough idea about quality of semen.

2.1.1 Volume of semen

Average volume of semen ejaculate in different breeds of bucks reported by various scientists is given below.

Mockel (1937) reported first time the volume of semen of Fawn White German breed of buck to be 0.57ml in the range of 0.1 to 1.25 ml.

Austin et al. (1968) recorded semen volume of 1.93 ml in Spanish goats.
Nimkar (1977) reported semen volume as 0.56±0.0 ml in Surti bucks.

Cetinkaya et al. (1980), Dunder et al. (1983), Loubser et al. (1983), Servinc et al. (1985) and Bakshi et al. (1987) reported semen volume in Angora bucks as 0.98(0.4 to 2.0 ml), 0.94±0.11 ml, 1.09 ml, 0.92 ± 0.15 ml and 0.95±0.03 ml respectively.

Keshavreddy (1983), Kishore and Rao (1983) reported semen volume as 0.72±0.022 ml and 0.86±0.092 ml respectively in nondescript bucks.

Greylinga and Grobbela (1983) recorded volume as 1.56 ml in Boer and 1.63 ml in Angora bucks.

Baviskar (1985), Parandekar (1987) and Puranik (1988) recorded semen volume as 1.15±0.05 ml, 0.591±0.022 ml and 0.56±0.033 (0.51 to 0.65) ml respectively in Osmanabadi bucks.

Puranik (1988) reported the semen volume in Osmanabadi crosses as 0.77 ± 0.023 (0.71 to 0.82) ml, values reported differed significantly within breeds but not within individual bucks.

Mendoza et al. (1989) collected semen by artificial vagina from 11 Angora goats, once or twice weekly, between April and July in two successive years. They observed the mean ejaculate volumes each year as 0.8 ± 0.30 and 0.98 ± 0.52 ml.

Bakshi (1991) recorded 0.65 ± 0.018 ml ejaculate volume of Osmanabadi bucks.

Ahmad and Noakes (1996) studied physical characteristics of semen for 12 months period in 10 post pubertal, young British male goats (7-19 months of age). They recorded that ejaculate volume decreased from 0.96 +/- 0.06 ml in October to the minimum value of 0.39 +/- 0.03 ml in April and 0.38 +/- 0.02 ml in July, after which there was a sharp increase to the highest value, 1.04 +/- 0.05 ml, in September.
Prado et al. (2002) conducted a trial on eight 2-yr-old Mexican bucks. They collected semen using an artificial vagina. They observed semen volume between $0.8 \pm 0.03$, $1.0 \pm 0.03$, and $0.9 \pm 0.02$ ml.

Silvestre et al. (2004) observed $0.43 \pm 0.05$ to $0.62 \pm 0.04$ ml semen volume in young Murciano-Granadina bucks.

Zamiri and Heidari (2006) studied semen characteristics of Iranian Rayini goats. They used artificial vagina to collect semen from 14 native Iranian Rayini goats, at 15-day intervals. They found semen volume between 1 and 1.4 ml.

Farshad et al. (2009) recorded $1.67 \pm 0.5$ ml ejaculate volume in Markhoz bucks.

Hanmante et al. (2009) in a study conducted on eighteen healthy Osmanabadi bucks which were randomly distributed among three groups (i) $T_1$: Grazing (ii) $T_2$: Stall fed and (iii) $T_3$: Semi-stall fed systems recorded semen volume, sperm motility, live/dead sperm count and sperm concentration at monthly interval from 6 to 15 months of age. Among the three treatments, $T_3$ treatment was found superior for getting good quality semen from Osmanabadi bucks.

Dorado et al. (2009) recorded total volume of ejaculate of $1.20 \pm 0.04$ ml in mature Florida bucks (2-year old).

Berlinguer et al. (2009) recorded $0.4 \pm 0.2$ to $0.8 \pm 0.5$ ml semen from adult Italian bucks.

El-Kon et al. (2010) obtained $1.1 \pm 0.03$ ml semen volume in Damascus bucks.

### 2.1.2 Colour and consistency of semen

Colour of semen gives approximate idea about quality of semen. The overall colour and consistency noted for different breeds of bucks by various scientists are given below.
Pale yellow colour of buck semen was reported by Mockel (1937) in German white breed.

Nimkar (1977) reported colour of Surti buck semen as yellowish creamy and thick in consistency.

Baviskar (1985) reported colour of Osmanabadi buck semen as creamy white.

Pandey et al. (1985) reported creamy coloured semen for Saanen bucks and yellowish creamy for Barbari bucks.

Bakshi et al. (1987) recorded colour of Angora and crossbred Angora buck semen as yellowish white with thick creamy consistency.

Parandekar (1987) reported colour of Osmanabadi buck semen as creamy white with thick consistency and the colour of crossbred buck semen as yellowish creamy with thick consistency.

Puranik (1988) reported colour of Osmanabadi and crossbred buck semen as creamy white with thick consistency.

Mendoza et al. (1989) collected semen by artificial vagina from 11 Angora goats, once or twice weekly, between April and July in two successive years. They noted the colour of semen as yellow, light yellow and white.

Bakshi (1991) recorded creamy white colour of semen from Osmanabadi bucks.

Ahmad and Noakes (1996) studied physical characteristics of semen for 12 months period in 10 post pubertal, young male goats (7-19 months of age) maintained under the naturally prevailing climatic conditions characteristic of the South of England (latitude N 51 degrees 46'). The physical appearance of the ejaculates varied from a yellow or whitish-yellow colour during September-December to a creamy-white colour during the remainder of the year.
2.1.3 Density of semen

Density of semen gives rough idea about semen. The watery and thinner density has poor quality semen. The density of different goat breeds semen noted by various scientists is given below.

Nimkar (1977) reported the density of Surti buck semen as 3.64 ± 0.08 in 4 point scale.

Baviskar (1985) noted the density of Osmanabadi buck semen as DDD (D) to DDDDD.

Parandekar (1987) observed the density of Osmanabadi bucks semen as 3.291 ± 0.055 (2.5 to 4) and that of crossbred bucks as 3.218 ± 0.044 (3 to 4) in 4 point scale.

Puranik (1988) reported the consistency of Osmanabadi bucks as DDD (D) and that of crossbred buck semen as DDD.

Bakshi (1991) recorded DD (D) to DDD density of semen from Osmanabadi bucks.

2.1.4 Hydrogen ion concentration (pH) of semen

The pH of semen of different goat breeds noted by various scientists is given below.

Knoblauch (1962) reported the pH of German white buck as 6.6.

Nimkar (1977) recorded the pH of Surti buck semen as 6.37± 0.7

Greylinga and Grobbela (1983) recorded pH of 6.4 to 7.02 in Boer and 6.51 to 7.02 in Angora bucks.
Baviskar (1985), Parandekar (1987) and Puranik (1988) reported the pH of Osmanabadi Buck semen as 6.25 to 6.8, 6.78 ± 0.002 and 6.55 ± 0.015 respectively.

In crossbred bucks, pH of buck semen was reported by Kho (1975), Prasad et al. (1986) and Parandekar (1987) and Puranik (1988) as 6.8, 6.68±0.002, 6.779 ± 0.016 and 6.49 ± 0.012 in Katjang x Jamnapari, Sannen x Barbari, Osmanabadi crossbreds and Osmanabadi crossbred respectively.

Bakshi (1991) recorded 6.62± 0.012, 6.58± 0.009, 6.60±0.009 and 6.60±0.008 pH of semen from Beetal, Osmanabadi, Sannen, Alpine crosses and Sannen crossbred bucks.

Zamiri and Heidari (2006) studied semen characteristics of Iranian Rayini goats. They observed that seminal fluid pH values were significantly lower from July to October (pH <6) than the values from November to December (pH >6.1)

### 2.1.5 Resazurin test

Resazurin was reported by Erb and Ehler (1950) as simple and rapid reduction test indicator for estimating fertilizing capacity of bull semen. The time required for semen to reduce resazurin to the pink endpoint compared with 60 to 90 day non-return rates (on a per semen sample basis) showed a highly significant correlation of -0.141 for - 371 semen samples.

Erb et al. (1952) found that when semen from 54 different bulls was used for resazurine test. The average time required for change in colour from blue to pink was 7.26±4.7 minutes.

Pathak et al. (1989) applied resazurin reduction test to the semen samples obtain from six triple crossbred bulls. The average reduction time from violet to pink in the crossbred bulls ranged between 136.66±19.88 to 478.16±72.57 seconds, and
average reduction time from pink to colourless ranged between 425.23±69.16 to 950.38±123.57 seconds.

Dart et al. (1994) studied total of 68 semen samples from 20 Limousin bulls which were collected using electro ejaculation. The concentration of motile and progressively motile sperm was determined. Assessment of the reduction of resazurin from blue to pink (≤3.5 min) allowed identification of 88% of the low and 94% of the high fertility potential samples. In addition, the reduction of resazurin from blue to white (<15 min) resulted in identification of 82% of the low and 76% of the high fertility potential samples. The modified resazurin reduction test is useful for determining the fertility potential of bovine spermatozoa.

Foote (1999) studied one hundred sixty-four ejaculates from 59 crossbred bulls. The resazurine reduction test (RRT) was significantly correlated with fertility when sperm numbers were standardized. The average RRT range (in minutes) from 3.3 to 7.3

Martin et al. (1999) studied the semen samples collected from 67 post-pubertal Rambouillet rams using electro-ejaculation. Only ejaculates containing a volume of ≥0.5 ml were tested (n = 121). Resazurin (3.1 μg) was added to each 0.5 ml sample and incubated for a period of 15 min or until a visible dye reduction from blue to white could be detected.

2.2 Microscopic evaluation of semen

Microscopic semen evaluation is routine technique in most of laboratories and semen bank. This method contains evaluation of mass activity, total concentration of sperm, sperm motility percentage, live sperm percentage and abnormal sperm percentage.
2.2.1 Mass activity

The mass activity of sperm indicates movement of sperm in masses. It is one of the most important tests for evaluating quality of semen. Mass activity of buck semen reported by various scientists is given below.

Nimkar (1977) reported mass-activity of Surti buck semen to be 3.6±0.11.

Sinha and Singh (1982) recorded the mass-activity of Black Bengal buck semen to be 4.44 and Saanen buck semen as 4.51.


In crossbred buck semen mass-activity was recorded to be 4.25 ± 0.07 in Jamnapari X Black Bengal, 4.18 ± 0.068 in Saanen X Barbari buck semen, 3.27 ± 0.066 in Osmanabadi cross buck semen and 3.645±0.081 in Osmanabadi crossbred buck semen by Singh et al. (1985), Prasad et al. (1986), Parandekar (1987) and Puranik (1988), respectively.

Bakshi (1991) recorded 3.75±0.04 mass activity in semen from Osmanabadi bucks.

Tuli and Holtz (1992) collected and evaluated semen samples from six healthy, mature Boer goat bucks. They have selected semen samples with mass activity greater than +3 for further processing.

Ahmad and Noakes (1996) reported that the mass motility of male goats at south England were higher during August to December than that in remainder of the year.

Sule et al. (2007) observed mass activity of grade 4 in African dwarf buck semen.

Farshad et al. (2009) recorded 4.07± 0.6 mass activity in Markhoz bucks.
2.2.2 Sperm concentration

Mishra and Sengupta (1965) and Dabas et al. (1982) reported sperm concentration of 4080 ± 450, 4324.99 ± 190.85 millions per ml in Jamnapari buck semen.

Austin et al. (1968) recorded sperm concentration of 2456.5 millions/ml of semen in Spanish breed of goat.

Nimkar (1977) recorded 3334.667 ± 209.80 millions sperms per ml of semen in Surti breed of Goat.

Cetinkaya et al. (1980), Loubser et al. (1983), Servinc et al. (1985) and Bakshi et al. (1987) reported sperm concentration in Angora Bucks as 3764, 1865.2, 3110 ± 37.00 and 2592.98 ± 86.34 million sperms per ml of semen respectively.

Singh et al (1982) recorded 2293.728 and 2792.44±80.22 millions sperm concentration in Jamanpari and Barbari bucks.

Baviskar (1985) reported total sperm concentration in Osmanabadi buck semen as 3143.45 ± 25.11 million per ml of semen.

Parandekar (1987) and Puranik (1988) recorded the sperm concentration in Osmanabadi buck semen as 3645.625± 54.019 and 3073 ± 24.528 million/ml of semen respectively.

In crossbred buck semen the sperm concentration was reported to be 3974.8 ± 130.8 (Katjang X Jamnapari), 2851.67 ± 211.728 (Black Bengal crossbreds), 2375.47 ± 7.095 (Saanen X Barbari), 2810.45 ± 96.77 (7/8 Angora cross), 3645.625 ± 54.019 (Osmanabadi cross) and 2763.15 ± 86.306 (Osmanabadi cross) million sperm per ml of semen respectively by Kho (1975), Singh et al. (1985), Prasad et al. (1986), Bakshi et al. (1987), Parandekar (1987) and Puranik (1988).

Mendoza et al (1989) collected semen by artificial vagina from 11 Angora goats, once or twice weekly, between April and July in two successive years. They
observed the sperm concentrations were $3.33\pm0.49$ and $2.94\pm0.45 \times 10^9$ ml of semen respectively.

Bakshi (1991), recorded 2924.64±24.17 millions/ml sperm concentration in semen from Osmanabadi bucks.

Ahmad and Noakes (1996) studied characteristics of semen for 12 months period in 10 post pubertal, young male goats (7-19 months of age) maintained under the naturally prevailing climatic conditions in the South of England. Sperm cell concentration per ml was lowest (3660 ± 16 million/ml), during November and highest (6560 ± 29 million/ml) during May.

Prado et al. (2002) in a trial on eight 2-yr-old bucks observed total sperm concentration as 4.4±0.15, 4.6±0.13, and 4.4±0.86 spermatozoa/ml

Silvestre et al. (2004) observed 2,223 ± 206 to 2,828 ± 149 millions/ml sperm concentration in young Murciano-Granadina bucks.

Khalifa and Saidy (2006) obtained semen from bucks twice a week via an Artificial Vagina with >2500 × 10^6 sperm/ml in Egypt during the breeding season of goats.

Dorado et al. (2009) recorded sperm concentration of 3.69±0.08×10^9 spermatozoa/ml in mature Florida bucks (2-year old).

Berlinguer et al. (2009) recorded 1787.06 ± 515.5 to 2373.9 ± 607.7 millions/ml sperm concentration in mature adult bucks in Italy.

Farshad et al. (2009) recorded 3.95±0.7 billion/ml sperm concentration in Markhoz bucks.

El-Kon et al. (2010) obtained 2718±32.60 millions/ml sperm concentration in semen of Damascus bucks.
2.2.3 Sperm motility percentage

A) Motility of sperm in neat Semen.

Cetinkaya et al. (1980), Servinc et al. (1985) and Bakshi et al. (1987) reported the initial sperm motility of Angora buck semen to be 86 (70-90), 83.49±3.63 and 80 to 90 per cent respectively.

Baviskar (1985) recorded 84.25 ± 0.70 (80-90) per cent initial sperm motility in Osmanabadi buck semen.

Pandey et al. (1985) reported 89.66 per cent initial sperm motility in semen from Saanen bucks.

Parandekar (1987) and Puranik (1988) recorded 70.20±2.773 and 70.05±2.01 percent initial motility in Osmanabadi buck semen.

Initial motility percent of crossbred buck semen was recorded to be 85.0 ± 0.76 (Katjang X Jamnapari), 80 to 90 (Angora cross) 71.458 ± 1.362 (Osmanabadi cross) and 75.03 ± 1.907 (Osmanabadi cross) by Koh (1975), Bakshi et al. (1987), Parandekar (1987) and Puranik (1988) respectively.

Bakshi (1991) recorded 79.28±0.95 percent initial sperm motility in semen of Osmanabadi bucks.

Dunner (1993) observed 72 % and 62 % sperm motility in fresh semen of two bucks.

Ahmad and Noakes (1996) reported that the initial motility of male goats at south England were higher during August to December than in remainder of the year.

Silvestre et al. (2004) observed 70 to 80 percent sperm motility in semen of young Murciano-Granadina bucks.
Khalifa and Saidy (2006) recorded 70% progressive sperm motility in Egypt during the breeding season of goats.

Kozdrowski et al. (2007) recorded 87.50 ± 4.62% sperm motility in fresh semen of French Alpine bucks.

Dorado et al. (2009) recorded 91.40 ± 0.65 percent sperm motility in fresh semen of mature Florida bucks (2-year old).

Farshad et al. (2009) recorded 86.13 ± 6.6 percent initial sperm motility in Markhoz bucks.

El-Kon et al. (2010) obtained 73.58±0.64 percent individual sperm motility in Damascus bucks semen.

**B) Pre/Post freeze motility of spermatozoa**

Deka and Rao (1986) in indigenous bucks from Andrapradesh recorded 64.0%, 66.92% and 63.65 % post thaw sperm motility when semen was frozen with 4, 6.4 and 9 % glycerol and 61.48 %, 65.05 % and 68.03 % in 1-, 3- and 5-h equilibrated semen, respectively.

Bakshi (1991) recorded 67.91±2.34 and 48.12±1.13 percent sperm motility in semen of Osmanabadi bucks in Tris extender at pre-freezing (5°C) and post-freezing (-196°C).

Tuli and Holtz (1992) noted 70±1.68 %, 65.25 ± 1.74 %, 44.50 ± 2.37 % and 43.75± 1.78 % sperm motility at Initial extension, equilibration, after 15 minutes of freezing and after 7 days of freezing respectively in Tris egg yolk glycerol dilutor in Boer goat semen.
Dunner (1993) in Spanish bucks observed 54% and 39% sperm motility at chilling of two bucks semen.

Tuli and Holtz (1994) recorded 69.0±2.4% and 38.5±2.7% progressive sperm motility prior to freezing and after thawing respectively in Boer goat semen using Tris egg yolk glycerol dilutor with one step freezing procedure.

Kundu et al. (2000) Observed that addition of glycerol (0.22 to 0.87 M) caused a dose-dependent increase of sperm motility recovery. The highest recovery of forward and total motility was (32 and 35%, respectively) at 0.87 M. Further increase of the glycerol concentration caused a marked decrease in motility. They also observed that like glycerol, dimethyl sulfoxide (Me(2)SO) and ethylene glycol also showed a dose-dependent increase in motility recovery as well as a biphasic curve of cryoprotection. At optimal concentrations, dimethyl sulfoxide (1.00 M) and ethylene glycol (1.29 M) were effective in recovering sperm motility to the extent of 20 and 13%, respectively. Thus these agents have markedly lower cryoprotection potential than glycerol.

Kundu et al. (2002) observed that Dextrans of 10, 40, 73, 173, 252, 500 and 2000 kDa offered maximum cryorecovery of forward motility to the extent of approximately 23%, 21%, 19%, 18%, 16%, 15% and 8%, respectively. Dextran also served as a significant cryoprotectant in the presence of glycerol (0.87mol⁻¹) and dimethyl sulphoxide (0.76 mol⁻¹). In the presence of the three cryoprotectants (Dextran, Glycerol and DMSO), recovery of sperm motility was as high as 58% (forward motility) and 60% (total motility).

Rizal et al. (2002) observed 70.00% motile sperm after equilibration in Garut ram semen.

Anel et al. (2003) observed 75.8 percent post thaw sperm motility in ram semen.

Eiman and Takato (2004) In a trial with Tris –citric acid-glucose-glycerol dilutor observed 59.0±2.3% and 46.0±2.9% sperm motility in Japanis goats. In another trial
using trehaloses- egg yolk- glycerol observed 73± 0.9 % and 57.0±1.8 % post thaw sperm motility in bucks. They concluded that the inclusion of egg yolk significantly improves sperm motility.

Khalifa and Saidy (2006) in Damascus bucks recorded 78.89±1.11; 80.56±0.56 and 81.67±1.18 percent sperm motility immediately post dilution at 1:2, 1:4 and 1:19 dilution rate. At dilution rate of 1:2, 1:4 and 1:19 they noted 31.11±4.23, 41.11±5.19 and 16.11±4.15 post thaw sperm motility 1 hour after freezing. They concluded that post-thaw sperm motility of semen diluted at a rate of 1:4 was significantly ($P < 0.01$) higher than that of diluted at other rates.

Kashiwazaki et al. (2006) studied effect of glycerol and dimethyl sulfoxide (DMSO) as cryoprotectants for Japanese White rabbit spermatozoa. In frozen thawed spermatozoa the rate of forward progressive motile spermatozoa in DMSO (37.8 ± 3.0%) was significantly ($P<0.05$) higher than in glycerol (17.0 ± 3.3%).

Selarnet et al. (2006) recorded 74.17 ± 2.04, 66.67 ± 4.08 and 42.50 ±5.24 percent sperm motility at dilution, pre-freezing and post thawing in Garut ram.

Kozdrowski et al. (2007) recorded 87.50 ± 4.62% sperm motility in fresh semen of French Alpine breed. They recorded reduced post thaw motility in two different protocols of semen dilution as 28.12 ± 6.51% and 22.50 ± 5.97%.

Sadia Afroz et al. (2008) in Black Bengal bucks observed that motility of sperm in Triladyl and Tris diluents ranged from 63.33% to 70.00 after equilibration in straws. Sperm motility of thawed semen varied from 38.33% to 43.33% and from 6.00% to−6.67% in Triladyl and Tris diluents, respectively.

Dorado et al. (2009) recorded 56.07 ± 1.32 percent sperm motility in Frozen–thawed semen of mature Florida bucks (2-year old).

Farshad et al. (2009) recorded 38.4 ± 0.9 (5% glycerol) and 41.2±0.5 (1.75% DMSO) percent sperm motility at 5°C in Markhoz bucks.
Umut and Daskin (2010) recorded 25.5%, 34.5%, 37%, and 49.5% percent post-thaw sperm motility in semen of Angora goats in different trials.

El-Kon et al. (2010) recorded 77.0±0.61%, 65.75±0.59%, 39.00±0.71% individual sperm motility after dilution, cooling and thawing respectively in semen.

Ari et al. (2011) in Tushin ram semen observed 25.0%, 30.8%, 7.5% and 14.1% post thaw sperm motility in different extenders.

2.2.4 Live sperm percentage

A) Neat semen

Austin et al. (1968) recorded 80% to 87 % live sperm percentage in semen of Spanish goats in two trials of semen collection.

Nimkar (1977) reported 82.87 ± 1.9 per cent live sperms in Surti buck semen.

Singh et al. (1985) reported the percentage of live sperms in Jamnapari buck semen as 75.30 ± 3.33%.

Baviskar (1985), Parandekar (1987) and Puranik (1988) observed live sperm percentage of Osmanabadi bucks semen as 84.22 ± 0.26, 72.022 ± 2.422 and 69.62 ± 1.967% respectively.

Bakshi et al. (1987) reported the live sperm percentage of Angora buck semen to be 86.69 ± 1.40%.

In crossbred buck semen the percentage of live sperm recorded were 86.37±1.00, 91.07 ± 0.544, 86.68 ± 1.10, 72.218 ± 1.230 and 73.11 ± 1.770% in Jamnapari X Beetal cross, Sannen X Barbari cross, Angora cross, Osmanabadi cross and

Bakshi (1991) recorded 72.56±1.03 percent live sperm in semen of Osmanabadi bucks.

Tuli and Holtz (1992) recorded 68.40±1.91 to 76.15±2.93 percent live spermatozoa after initial extension of Boer bucks semen with different dilutors.

Chauhan et al. (1994) noted 90 % normal live spermatozoa in fresh semen ejaculate in Jamnapari bucks.

Ahmad and Noakes (1996) reported that the percentage of dead spermatozoa in semen of male goats at South England were highest during May.

Nur et al. (2003) undertook a study to assess the effectiveness of different staining methods, Eosine-Nigrosin (EN), Giemsa and double staining method composed of EN combined with Giemsa (EN-G) for the detection of dead spermatozoa in fresh semen from Saanen goats. They observed no significant differences in the percentage of dead spermatozoa between the semen stained with EN and EN-G.

Oyeyemi et al. (2001) recorded 96.57 % live spermatozoa in semen collected from West African Dwarf goats aged between 2-4 years and weighing between 16-20 kg.

Silvestre et al. (2004) observed 70 to 80 % live sperm percentage in young Murciano-Granadina male goats.

Zamiri and Heidari (2006) observed that percent live sperm were significantly higher during the summer months in Iranian Rayini bucks. The average percent live sperm during the sampling period varied between 60% and 78%. The total number of live and normal sperm in the ejaculate during the sampling period varied from 1000 to 2500 million.
Farshad et al. (2009) recorded 88.53 ± 7.3 percent live sperm percentage in Markhoz bucks. In the same bucks they recorded 49.1 ± 1.6 and 50.6 ± 0.7 percent live sperm percentage with 5 % glycerol and 1.75 % ethylene glycol dilutor at 5°C temperature.

B) Pre/post freeze live and dead sperm percentage

Bakshi (1991) recorded 65.41±2.50 and 41.94±3.15 percent live sperm in semen of Osmanabadi bucks in Tris extender at pre-freezing (5°C) and post-freezing (-196°C) stage.

Bakshi (1991) recorded average live sperm percentage of Beetal, Saanen, Alpine crossbreds and Saanen crossbreed semen preserved at 5°C in tris extender as 67.00±4.21, 59.83±2.86, 60.25±2.52 and 58.50±2.91 respectively. The corresponding values of semen preserved at -196°C in tris extender were 51.23±2.22, 43.28±1.67, 40.03±1.17 and 46.09±1.41 respectively.

Tuli and Holtz (1992) in Boer bucks recorded live sperm percentage as 54.85±3.71 to 60.20±3.35 after equilibration ; 26.40±2.78 to 34.25±2.50 after 15 minutes of freezing and 26.60±2.22 to 30.90±2.66 after 7 days of freezing in Boer goat semen in different dilutors. They opined that semen samples when extended with Tris yolk glycerol showed significantly (P<0.01) higher live spermatozoa when extended with the other zwitterions buffer-based extenders.

Tuli and Holtz (1994) recorded 74.4±3.0 and 42.3±2.9 percent live spermatozoa prior to freezing and after thawing of semen of Boer goat respectively.

Rizal et al. (2002) observed 77.00% live sperm after equilibration in Garut ram semen.

Anel et al. (2003) observed 31.3 percent post thaw dead sperm percent in ram semen.
Fernandez-Santos et al. (2006) in Red Deer for epididymal spermatozoa at pre freeze stage with 6% glycerol and 6% ethylene glycol dilutor recorded 60-70% and 50-60% live sperm percentage respectively.

Selarnet et al. (2006) recorded 82.83 ± 1.60, 75.00 ± 3.52 and 55.67±3.08 live sperm percent at dilution, pre-freezing and post thawing in Garut ram.

Farshad et al. (2009) recorded 91.3 ± 0.7 percent normal acrosome sperm percentage in Markhoz buck semen. In the same bucks they recorded 91.3 ± 0.7 and 88.4 ± 0.7 percent intact acrosome sperm percentage with 5 % glycerol and 1.75 % ethylene glycol dilutor at 5°C temperature.

Umut and Daskin (2010) recorded 36%, 52%, 54% and 61% post-thaw dead sperm in semen of Angora goats in different trials.

2.2.5 Abnormal sperm percentage

A) Neat semen

Austin et al. (1968) recorded 93% to 94 % normal sperm percentage in semen of Spanish goats in two trials of semen collection.

Nimkar (1977) recorded 8.47 ± 1.73 per cent abnormal sperms in Surti buck semen.

Cetinkaya et al. (1980), Loubser et al. (1983) and Servinc et al. (1985) reported abnormal sperms in Angora bucks as 23, 19.84 and 20.6 percent respectively.

Saxena and Tripathi (1980) studied the seminal attributes of Jamnapari bucks. Sperm head abnormalities, mid piece abnormalities, tail abnormalities and total sperm
abnormalities recorded were 0.88, 4.83, 1.13 and 6.84 per cent respectively. The free sperm heads and swollen head abnormalities recorded were 0.80 and 2.70 per cent respectively.

Sinha and Singh (1982) reported the percentage of abnormal sperm in Saanen buck semen as 6.20 per cent.

Baviskar (1985) reported abnormal sperm percentage as 5.39 ± 0.38 in Osmanabadi buck semen.

Parandekar (1987) and Puranik (1988) reported 4.583 ± 0.345 and 6.03 ± 1.172 per cent abnormal sperms in semen of Osmanabadi buck.

Keshavreddy (1983) recorded the loose sperm head, proximal proplasmic droplet, distal proplasmic droplet, bent tail and total sperm abnormalities as 1.63 to 2.18, 0.068 to 0.22, and 1.51 to 2.80, 3.53 ± 6.23 and 6.73 to 11.00 per cent in local bucks ageing more than 2 years.

Bakshi (1991) recorded 6.06±0.14 percent total sperm abnormality in semen of Osmanabadi bucks.

Roca et al. (1992) evaluated seasonal variations of semen quality in male goats inhabiting in Mediterranean region. They found distal cytoplasmic droplets and acrosomal damage were the predominant abnormalities. All types of sperm abnormalities studied showed significant (P<0.01) seasonal variation. The poorest quality semen was collected during winter and spring. The proportion of abnormal spermatozoa was well within the accepted range for normal fertility.

Ahmad and Noakes (1996) reported that the percentage of morphologically abnormal spermatozoa in semen of male goats at south England were highest during May.

Zamiri and Heidari (2006) studied semen characteristics of Rayini male goats of Kerman province in Iran between July to December 2000. Percent normal sperm were significantly higher during the summer months. The average Percent abnormal sperm during the sampling period varied between 7 and 13 respectively.
B) Pre/Post freeze abnormal sperm percentage

On scanning, available literature for total sperm abnormality post freezing in bucks semen was scanty especially for DMSO and ethylene glycol, hence references from other species were also reviewed.

Bakshi (1991) recorded 8.33 and 9.18 percent abnormal sperm percentage in semen of Osmanabadi bucks in Tris extender at pre-freezing and post-freezing (-196°C) stage.

Bakshi (1991) recorded average abnormal sperm percentage of Beetal, Saanen, Alpine crossbreds and Saanen crossbreed semen preserved at -196°C in tris extender as 8.88, 9.63, 8.30 and 9.60 respectively.

Azerêdo et al. (2001) evaluated morphology of fresh and frozen goat spermatozoa with or without seminal plasma. Semen samples were diluted in Tris solution, before and after thawing. The results showed differences (P<0.01) for minor defects in the presence or absence of seminal plasma, for both fresh and frozen samples. They concluded that the photoperiod probably decreased the testosterone level, contributing negatively to the high percentage of sperm abnormalities, mainly damaged membranes.

Rohilla et al. (2005) studied 20 semen ejaculates collected at weekly intervals from 3 Murrah buffalo bulls. Each ejaculate was divided into three equal parts. One part was added with 6.8% glycerol, the second part with 5% ethylene glycol and the third part with 3% propylene glycol. Sperm abnormalities during pre- and post-freezing and thawing of semen were less (6.50±0.04 and 9.30±0.03%) in 5% ethylene glycol than in 6.8% glycerol (7.00±0.05 and 10.30±0.07%). It was concluded that ethylene glycol gives the satisfactory values for post thaw motility, live spermatozoa and intact acrosome.

Nandre (2007) in Surati bulls observed on preservation of semen in Tris glycerol dilutor at pre-freeze and post-freeze stages, the abnormal sperm count was 3.91 ± 0.40% and 4.91 ± 0.49%. In winter the abnormal sperm count was 3.72 ± 0.52% and 3.94 ± 0.50% and in summer it was 4.11 ± 0.61% and 5.88 ± 0.79%.
Batista et al. (2010) observed in Majorera Bucks that sperm abnormality along with other semen parameters was not changed in Tris based dilutor when preserved at 4°C.

Ari et al. (2011) in Tushin ram semen observed 49.50 to 65.7 percent post thaw sperm abnormality in different extenders.

**2.3 Sperm function test**

**2.3.1 Plasma membrane integrity (Hypo-Osmotic Swelling Test)**

Hypo-osmotic swelling test is one of the procedures used in the evaluation of the reaction that the functional integrity of plasma membrane of spermatozoa reveals under hypo-osmotic conditions (Jeyendran et al. 1984). HOS test investigates whether the spermatozoon membrane is functional or not. Therefore, HOS test is used as a method complementing the routine sperm analysis (Nie and Wenzel, 2001).

As in other species of domestic animals (Correa et al., 1997; Neild et al., 1999), goat spermatozoa had a similar pattern of swelling when exposed to a hypoosmotic medium. It is proposed that under such conditions, biochemically-active sperm cells, with intact membranes, absorb water and swell increasing in volume to establish equilibrium between the fluid compartment within the spermatozoa and the extracellular medium. It culminates into a spherical expansion of the cell membrane covering the tail, thus forcing the flagellum to coil inside the membrane. Tail coiling begins at the distal end of the tail and proceeds towards the midpiece and head as the osmotic pressure of the suspending media is lowered (Jeyendran et al.1984).

The review pertaining to buck semen for HOST was scanty; hence references of other species were also obtained.
Rasul et al. (2001) carried out a study to identify suitable buffer for cryopreservation of buffalo bull semen. The post thaw HOS positive sperm percent in citrate, TCA, TEST, and HEPES was 45.2±1.2, 32.6±3.8, 41.4±5.2 and 40.8±3.9 respectively.

Azeredo et al. (2001) evaluated plasma membrane integrity of fresh and frozen goat spermatozoa with or without seminal plasma. Semen samples were diluted in. Plasma membrane integrity was significantly reduced by the freezing process, whether seminal plasma was present or absent.

Rasul et al. (2001) pooled semen ejaculates from 4 buffalo bulls (n 5), diluted in tris-citric acid extender, cooled to 4°C over 2 hours, equilibrated at 4°C for 4 hours, dispensed into 0.5-mL straws, and frozen in a programmable cell freezer before plunging into liquid nitrogen. Frozen semen was thawed at 37°C for 15 seconds. Sperm plasma membrane integrity was 80.2± 3.9% after dilution, reduced (P ≥0.05) to 60.4±5.6% after equilibration, and then to 32.6±3.8% after freezing and thawing. Spermatozoa with normal acrosomes did not change due to dilution, cooling, or equilibration (73.2±2.4%) and were the lowest (P≥.05) after freezing and thawing (61.8±2.4%)

Henry et al. (2002) in stallion obtained post thaw-HOS% in 5% ethylene glycol dilutor was 14.7±11.4 %.

Mantovani et al. (2002) compared glycerol (G) at customary concentrations and ethylene glycol (EG) at different concentration as cryoprotectants for stallion semen in a skimmed milk (SM) extender with manual and computerized analysis of hypo osmotic swelling (HOS) test. The pre freeze HOS % for 3% ethylene glycol was 88.2±1.9% and overall post thaw HOS % was 50.9 % for 3% ethylene glycol.

Arangasamy et al. (2005) in Murrah buffalo bulls recorded 66.4±0.65 % HOS positive sperm values for post thawed semen.

Fonseca et al. (2005) tested HOST technique in Saanen and Alpine bucks. They studied the best hypo osmotic solution (HS) for testing membrane integrity in fresh goat semen. They used sodium citrate and fructose based solutions (S) with the different osmolarities (mOsm/l) like 50 (S1), 75 (S2), 100 (S3), 125 (S4), 150 (S5), 175
(S6), 200 (S7), 250 (S8), 290 (S9) and 300 mOsm/l (S10). The respective percentages of spermatozoa with coiled tails (coiled plus strongly coiled) in the ten solutions listed above were 34.1, 38.8, 45.3, 51.5, 46.8, 42.8, 38.2, 29.0, 19.4 and 23.1%. Results suggest that the 125 mOsm/l solution would be best for use in HOST in fresh goat spermatozoa.

Li et al. (2005) studied the cryoprotective effect of 11 extenders in Cynomolgus Monkey. The neat semen HOS% was 83.27±5.83% and post thaw HOS% in Tris dilutor containing ethylene glycol was 54.63±7.73%.

Nur et al. (2005) assessed the relationships between sperm membrane integrity, evaluated by the hypo-osmotic swelling (HOS) and water tests in Saanen bucks. For that purpose, a total of 60 fresh ejaculates from 6 Saanen bucks were evaluated. The mean percentage of swollen tail spermatozoa obtained with the HOS and water tests were 53.7±11.3% and 49.8±9.7%, respectively.

Fernandez-Santos et al. (2006) observed that in Red Deer epididymal spermatozoa pre freeze HOST % in 6% glycerol and 6% ethylene glycol were 80-85 % and 75-80% respectively. In post freezing the values were 60-65% and 40-50%, respectively.

Kashiwazaki et al. (2006) studied effect of glycerol and dimethylsulfoxide (DMSO) as cryoprotectants for Japanese White rabbit spermatozoa. In frozen thawed spermatozoa, sperm plasma membrane integrity in DMSO (35.9 ± 3.3%) was significantly (P<0.05) higher than in glycerol (17.0 ± 2.6%).

Leboeuf et al. (2006) in an experiment exposed buck spermatozoa to a wide range of osmotic pressures and then evaluated the reacted sperm cells by flow cytometry and finally modeling the sperm cell responses. Semen samples were diluted in skim milk or NPPC (native phosphocaseinate) extenders, and stored at 4ºC for 3 days. At D0 and D3 aliquots from each ejaculate (n=12) were submitted to seven hypo osmotic solutions varying from 230 to 10mOsm/kg. Spermatozoa exhibited a high variation from 230 to 10mOsm/kg which was considered as a dose-response curve. They had noted 36.8 to 72.9 percent membrane damaged spermatozoa by HOS test in different dilutors.
Salvador et al. (2006) in Murciano-Granadina bucks observed 48%, 31 to 34% and 21 to 31% HOS sperm percentage in fresh semen, diluted semen and in semen stored at 5°C respectively.

Selarnet et al. (2006) recorded 80.67±1.21, 72.00±1.97 and 54.17±2.71 percent plasma membrane integrity at dilution, pre-freezing and post thawing in Garut ram.

Temwong et al. (2006) studied the semen characteristic from five swamp buffalo bulls. The HOS positive sperm % at rainy, cool and summer season was 67.94±23.68, 70.67±15.09 and 71.92±12.03 respectively.

Gang Zhang et al. (2007) evaluated the plasma membrane integrity in fresh and frozen goat spermatozoa. They recorded 64.26 ± 7.03 and 46.25±2.69 % plasma membrane integrity in fresh and frozen semen respectively.

Gupta and Misra (2007) conducted an experiment to assess suitability of buffalo semen refrigerated up to 72h for use in artificial insemination employing hypo-osmotic swelling test (HOST) in addition to sperm motility, livability, and abnormality. HOST percentage declined from 65 in neat semen to 53 in refrigerated semen at 72h. The corresponding value for motility were 78 and 37 % respectively.

Salvador et al. (2007) evaluated Murciano Granadina buck semen using HOST. HOST was performed only at 0 hours incubation time, by adding an aliquot of semen (10 ul) in 1000 ul of HOST solution. They performed assessment after one hour incubation at 37°C under phase contrast microscope by scoring percentage of spermatozoa with swollen tail. They found that in milk extender the sperm membrane integrity (HOST) was better.

Quintela et al. (2010) observed 90.6 ± 0.1, 80.3 ± 0.3 and 53.4 ± 0.7 percent integrity of plasma membrane of fresh, cooled and frozen-thawed canine sperm.
2.3.2 Acrosome Integrity

Chauhan *et al.* (1994) in Jamnapri bucks found that in fresh ejaculated semen over 90% of the spermatozoa had normal head and acrosome morphology. In Giemsa stain goat sperm structure revealed significant (P < 0.01) damage to acrosome on freezing which varied between 38 to 43% in different diluents. The ultra structural changes detected in frozen goat sperm was protrusion at the anterior cap, broken tail, swelling of acrosome, and loss of acrosomal contents.

Chandler *et al.* (1998) collected, processed, and frozen semen from five dairy bucks for two successive years for use in artificial insemination programs. Semen was evaluated for percent post thaw intact acrosomes, they noted 46.4±1.26 to 65.5±1.26 % intact acrosome. Significant high correlations were found between: percent progressive motility and percent intact acrosomes.

Reyes *et al.* (2002) recorded 97.2 per cent acrosomal intactness in fresh boar semen. They also recorded 45.5 to 16.8 per cent acrosomal intactness in frozen boar semen.

Anel *et al.* (2003) observed 25.4 percent post thaw acrosomal damaged sperm in ram semen.

Eiman and Takato *et al.* (2004) in Japanese bucks spermatozoa noted 69.0±1.2 and 78.0 ±3.3 percent intact acrosome in trehalos dilutor with or without egg yolk addition prior to freezing. In the same group they had noted 49.0±3.0 and 74.0±3.9 intact acrosome post thawing respectively. In another experiment they found 60%, 80 % and 80 % intact acrosome in TCG-G (Tris citric acid glucose- glycerol), TCG-Y (Tris citric acid glucose- egg yolk) and TCG Y+G (Tris citric acid glucose- egg yolk +glycerol) dilutors respectively. They concluded that neither glycerol nor egg yolk alone affect the proportion of intact acrosomes; the combination of the two significantly reduced the proportion of acrosome-intact spermatozoa.
Li et al. (2005) studied cryoprotective effect of 11 extenders in Cynomolgus Monkey. The neat acrosome integrity (%) was 91.09±5.05 and post thaw acrosome intact (%) was 81.92±5.87 in Tris dilutor containing ethylene glycol.

Rohilla et al. (2005) in Murrah buffalo bull semen freezing used 6.8% glycerol, 5% ethylene glycol and 3% propylene glycol as cryo-protectant. The semen was placed in mini-German straws and after 4 h of equilibration at 5°C; it was frozen in liquid nitrogen. Semen quality was evaluated at various stages of semen processing by recording the percentage of intact acrosome. Semen added with and frozen in 6.8% glycerol resulted higher intact acrosome (77.50±0.26) followed by semen extended with 5% ethylene glycol (73.70±0.34) and 3% propylene glycol (65.90±0.61).

Martins-Bessa et al. (2006) observed in dogs that the post thaw acrosome intact sperm percentage in dilutor with 5% ethylene glycol was 37.8±11.0 and dilutor with 4% ethylene glycol was 36.1±11.9.

Fernandez-Santos et al. (2006) studied that in Red Deer at pre freeze stage the acrosome intactness for 6% glycerol and 6% ethylene glycol was 80-90% and 70-80% respectively. In post freezing for 6% glycerol and 6% ethylene glycol the motility was 60-70% and 50-55 % respectively, the acrosome integrity were 60-65% and 40-50% respectively.

Rafiqul Islam et al. (2006) evaluated semen of crossbred (Beetal × Assam Local) bucks. They observed that in fresh semen acrosome intactness was 73.3%.

Salvador et al. (2006) observed 70.0± 1.10 and 64±1.10 percent acrosome integrity in washed and unwashed buck semen respectively.

Gang Zhang et al. (2007) recorded acrosome integrity rate between 80.77±10.70 and 58.42±18.05 in fresh and frozen bucks semen from china.

Sheshtawy et al. (2008) studied ejaculate from five buffalo bulls. The average Motility (%), Membrane integrity (%) and Acrosome integrity (%) were 60.50±1.74, 71.10±1.57 and 65.30±1.73 respectively after cooling and 32.50±1.53, 41.10±1.66 and 50.30±2.89 respectively after thawing.
Dorado et al. (2009) assessed 110 ejaculates of goat semen in order to determine the acrosome integrity. They observed 90.33± 0.46 and 47.08± 1.43 percent acrosome membrane integrity in fresh and frozen thawed semen samples.

El-Kon et al. (2010) recorded 91.23 ±0.84%, intact acrosome in fresh semen, After dilution, cooling and thawing they had recorded 91.0±0.92%, 71.80±0.81%, 54.25±0.82% intact acroosome respectively in Damascus bucks semen.

2.3.3 DNA integrity test

2.3.3.1 DNA integrity by Acridine orange test

After scanning available literature the references regarding DNA integrity test by Acridine orange test for buck semen evaluation were scanty therefore literature of other species was reviewed.

Kosower et al. (1992) checked the effect of DDT on human spermatozoa. They obtain green fluorescence 71 ± 5.3% and 85 ± 4.9% respectively for treatment and control group while red fluorescence percentage was 5 ± 2.8% and 1% respectively for treatment and control group by AO method. While in hamster the control group showed 100% green fluorescence sperm.

Eggert-Kruse (1996) observed the percentage of human spermatozoa showing green fluorescence by Acridinee orange (AO) staining method 5 to 81%.

Spano et al. (1999) studied 19 healthy men aged between 30-38 years. The percentage of green fluorescent heads after AO staining was 71.7 ± 7.7% (range 59–89%).

Erenpreiss et al. (2001) carried out the tests on sperm from 40 fertile and infertile men to evaluate 2 methods of DNA denaturation in situ using Acridinee orange stain by Tejada's Method and Rigler-Roschlau Method. The percentage of
damaged (immature) sperm cell in fertile and infertile group was 23.9±7.5, 52.1±21 by Tejada’s Method and 17±3.8 and 30.2±6.8 by Rigler-Rosclau Method.

Chohan et al. (2004) studied DNA damage in human sperm by Acridine orange staining with different fixatives. Frozen-thawed semen samples showed increased damage to sperm DNA under both Carnoy’s (fresh: 10.9±1.3%; frozen: 30.8±2.9%); and Diff Quik fixation (fresh: 6.2±0.8; frozen: 17.1±2.5%)

Chohan et al. (2006) evaluated chromatin integrity in sperm from 60 infertile men and 7 fertile donors. DNA fragmentation in sperm evaluated by AOT did not differ (P > .05) between infertile men (31.3 ± 2.4) and donors (32.7 ± 4.8). AOT showed extreme variations for sperm DNA fragmentation in semen from both infertile men and donors.

Khalifa et al. (2006) assessed semen samples of 30 infertile and 30 fertile men. The mean sperm concentration, motility and normal morphology were significantly higher in the fertile subjects. The mean percentage of green fluorescent sperm in Acridine orange test (AOT) was 62.4 ± 28.8% and 53.8 ± 24.3% in the fertile and infertile subjects, respectively (Approximately 37 to 46 percent DNA damaged sperm).

Martins et al. (2007) obtained semen samples from a Nellore bulls. They used the Acridine orange test (AOT) to assess the DNA status of sperm cells preserved with differently lypholization media. The AOT indicated 0.5% DNA damage.

Thuwanut et al. (2007) studied DNA integrity of frozen-thawed epididymal cat spermatozoa (n=8) stained with 1.0% Acridine orange (AO) in distilled water in three different extenders; egg yolk tris extender I (EE-I), egg yolk tris extender I with cysteine (EE-C), egg yolk tris extender I with vitamin E (EE-Ve). Mean ± SD of DNA integrity was 86.7±5.2, 89.1±3.4 and 88.1±4.3% respectively in post thaw sperm after 0 hr.

Ali et al. (2008) concluded from various experiments that there was no significant difference in the percentage of spermatozoa with intact DNA between the frozen and fresh semen of bucks.
Chanapiwat Panida et al. (2010) studied DNA damage in frozen thawed Boar semen using Acridine orange (AO) staining. They recorded on average, DNA damage as 0.5%. DNA damage varied among the boars from 0.0% to 4.0%.

El-Nattat et al. (2011) recorded 94.00±1.510 and 91.63±0.94 percent DNA integrity after two hours of cooling of tris extended semen in rabbits. (Approximately 6 % DNA damage).

2.3.3.2 DNA integrity test by single gel electrophoresis (Comet Assay)

After scanning available literature the references regarding DNA integrity test by single gel electrophoresis (Comet Assay) for buck semen evaluation were scanty therefore literature of other species was reviewed.

The comet assay or single-cell gel electrophoresis assay has been used to detect DNA damaged in variety of cell type including spermatozoa. The comet assay has been used to detect damage induced by oxidation and to investigate potential damage as result of handling practice (Boe-Hansen et al. 2005).

In Comet assay individual cells are embedded in a thin agarose gel on a microscope slide. All cellular proteins are then removed from the cells by lysing. The DNA is allowed to unwind under alkaline/neutral conditions. Following the unwinding, the DNA undergoes electrophoresis, allowing the broken DNA fragments or damaged DNA to migrate away from the nucleus. After staining with a DNA-specific fluorescent dye such as ethidium bromide or propidium iodide, the gel is read for amount of fluorescence in head and tail and length of tail. The extent of DNA liberated from the head of the comet is directly proportional to the amount of DNA damage. The Comet Assay can be used to detect DNA damage caused by double strand breaks, single strand breaks, alkali labile sites, oxidative base damage, and DNA cross-linking with DNA or protein. The Comet Assay is also used to monitor DNA repair by living cells.
In domestic Farm animals, the alkaline Comet assay has been applied on stallion semen (Linffor and Meyers 2002), also on boar semen (Fraser. and Strzezek 2004), on bull semen (Boe-Hansen et al. 2005) and on buffalo semen (Nandre 2007).

Aravindan et al. (1997) reported susceptibility of mammalian sperm DNA to low pH or heat-induced denaturation in situ. The percentage of Comets observed for these samples ranged from 5 to 95%; these data correlated strongly with the percentage of sperm with increased DNA denaturability (R=0.973; P < 0.001).

Hughes et al. (1999) used Comet and ELIZA for DNA integrity in human spermatozoa. There is no significant difference in Comet (81.3% undamaged sperm) and ELIZA (80.8%) in these two method.

Donnelly et al. (2001) used alkaline gel electrophoresis (Comet assay) to evaluate DNA damage in fertile male. There was no significant difference in DNA damaged between fresh and frozen semen in human spermatozoa (84.21 and 83.17% undamaged DNA respectively.)

Singh et al. (2003) reported effects of age on DNA double-strand breaks and apoptosis in human sperm. Age correlated with an increasing percentage of sperm with highly damaged DNA (range: 0-83%) assessed by using the neutral microgel electrophoresis (Comet) assay and tended to inversely correlate with percentage of apoptotic sperm (range: 0.3%-23%).

Ding et al. (2004) studied sperm quality of males living in cold area and investigated the effects on male reproductive function working under cold area. Semen routine analysis showed that the semen parameters of the males working in cold area were within normal range, but level I Comet cell percentage in SCGE increased significantly, which was 4.4%, compared to the contrast group (1.9%) with significant difference. Long-term exposure to cold could induce sperm DNA injury, but did not affect sperm quality.

Fraser and Strzezek (2004) reported the use of comet assay to assess DNA integrity of boar spermatozoa following liquid preservation at 5°C. The percentage of
Comet-detected spermatozoa with damaged DNA was 2.90+0.28, 3.05+0.32, and 2.95+0.30 % in K-3, K-3/LPFh and K-3LPFo respectively.

Isachenko et al. (2004) observed no significant differences in the DNA integrity of prepared spermatozoa related to the freezing method or presence of a cryoprotectant ($P > 0.5$). The proportions of sperm showing undamaged DNA were 85.09 and 89.51%, for fresh sperm treated or not treated with the cryoprotectant respectively, 84.62 and 83.53%, for the slowly frozen sperm with or without cryoprotectant respectively, and 87.24 and 84.66%, for the vitrified sperm with or without cryoprotectant respectively.

Isachenko et al. (2004) reported that there was no effect of freezing method on DNA integrity on human spermatozoa. The DNA integrity in neat, quick freezing sperm and slow freezing sperm were 95%, 88% and 91% respectively.

Boe-Hansen et al. (2005) reported DNA integrity in sexed bull sperm assessed by Neutral Comet Assay (NCA) and Sperm Chromatin Structure Assay (SCSA). The NCA showed that the conventional samples had a higher tail moment (TM) ($P < 0.01$) than the sorted samples and that there was no difference between the samples in tail length (TL) ($P = 0.36$). It was concluded that NCA and SCSA can be used in assessing DNA integrity in bovine sperm and that cell sorting by flow cytometry improves the integrity of the sperm cell population. The head intensity was 98.51, 96.74 and 97.63 in bull 1, 2 and 3 respectively.

Fraser et al. (2006) studied sperm head DNA damage in bulls. Result indicated that the sperm genome was also well preserved after cryopreservation. Using a modified protocol of the comet assay found that the percentage of head DNA (DNA intactness) of fresh semen (81.6%) was not significantly decreased after cryopreservation (80.7%; n=12). It means that after cryopreservation about 1% head DNA was damaged.

Li et al. (2007) reported damage to chromosomes and DNA of Rhesus monkey sperm following cryopreservation. They analyzed fresh and frozen-thawed sperm for DNA damage using the comet assay. The percentage of fresh sperm with damaged DNA in ejaculated semen was 0 to 2.7 % ($n = 5$). Conventional cryopreservation and storage in liquid nitrogen caused DNA damage in 25.3 to 43.7 % of sperm, and when
sperm were frozen without cryo-protectants, 52.7 to 92.0 % of thawed sperm had DNA damage.

Nandre (2007) studied DNA damage in buffalo bull spermatozoa. The Mean Comet percentage of spermatozoa under experiment was 5.30 ± 0.94%. In winter, it was 3.00 ± 0.55% and in summer, 7.60 ± 1.64%. Comet percentage of spermatozoa was significantly higher (P < 0.05) during summer. On preservation of semen at pre-freeze and post-freeze stages, the Comet percentage was 6.97 ± 1.13% and 9.11± 1.31%. In winter, it was 2.88 ± 0.60% and 4.61± 1.07% and in summer it was 11.05 ± 1.70% and 13.61 ± 1.90%.

Ibrahim et al. (2008) observed 1 to 5 % DNA damage in an experiment in Boer male goats.

Berlinguer et al. (2009) observed 10.2 ± 1.8 to 14.9 ± 1.8 percent DNA damage by comet assays in frozen thawed spermatozoa collected from three adult male goats.

2.4 Enzyme leakage test

After scanning available literature the references regarding enzymes especially for Transaminases, Phosphatases and lactos dehydrogenase test for buck semen evaluation were scanty therefore literature of other species was also reviewed.

2.4.1 Transaminase (GOT/GPT)

Murdoch and white (1968) reported that if a particular enzyme is present in whole semen, it occurs in both spermatozoa and seminal plasma. They also observed that enzymes such as GOT, ACP, AKP and LDH were leached out of ram and bull spermatozoa by sudden cooling or deep-freezing. They recorded 293±50 595±113. 2400±340, 90±159 and 16800±30300 unit/ml GOT in ram, bull, dog, rabbit and human whole semen respectively.
Chaunhan and Srivastav (1973) studied enzyme composition of buffalo seminal plasma and reported that GOT value averaged 166.72 ± 40.08 units/ml of seminal plasma in buffalo bulls.

Zahariev et al. (1974) in their studies on transaminase activity of 13 Bulgarian Brown bulls semen found that the GPT enzyme activity was 17.2 units in spermatozoa and 13 units in seminal plasma before freezing, whereas 12.6 and 11.6 units, respectively after freezing to -196 °C.

Kaker and Arora (1976) studied semen transaminases of young Holstein Friesian X Hariana and Brown Swiss X Hariana cross – bred bulls. The mean seminal GOT and GPT values ranged from 316 to 704 and 18 to 68 Bu/ml, respectively.

Singal et al. (1976) reported a wide variation in seminal GOT and GPT levels among the breeds and within the breed crosses of Hariyana with Jersey, Holstein Friesian or Brown Swiss. The overall mean GOT and GPT was 101.50 ± 12.99 and 60.00± 13.54 BU/ml of neat semen.

Naifornita et al. (1977) assayed 94 ejaculates from bulls during three winter months namely December, January and February. The values of alanine transaminase (GPT) activity averaged 24.7, 16.3 and 18.5 units/ml and of aspartate amino transferase (GOT) activity 90.0, 44.3 and 107.5 units/ml, respectively for the three months, the difference between months being highly significant for both the enzymes.

Varshney et al. (1978) determined transaminases, in seminal plasma of six Murrah buffalo bulls semen, the GPT activity averaged 20.4± 0.47 units/ml and the GOT activity 83.5 ± 3.00 units/ml of seminal plasma.

Jain (1979) studied the effect of the deep freezing on release of the GOT from buffalo spermatozoa. He found the significant difference in GOT values pre-freeze and post-freeze, which decreased from 351.28 ± 69.01(in pre freeze) to 201.74 ± 20.74(in post freeze) units/mg protein.
Tuli et al. (1986) studied the effect of different extenders on GOT and GPT release from frozen Murrah buffalo semen. Sixty semen samples from 6 Murrah bulls, frozen in tris yolk glycerol (TYG), citric acid whey glycerol (CAWG) and egg yolk glucose sodium bicarbonate glycerol (EYGSBG) extenders revealed highly significant differences in GOT and GPT release among extenders and between bulls at different stages of freezing. The details as under.

<table>
<thead>
<tr>
<th>Diluents</th>
<th>Stages of freezing and enzyme released (Units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TYG</td>
<td>After first extension</td>
</tr>
<tr>
<td></td>
<td>GOT</td>
</tr>
<tr>
<td></td>
<td>51.71±4.91</td>
</tr>
<tr>
<td></td>
<td>(31.95)a</td>
</tr>
</tbody>
</table>

first extension and 15 minutes after freezing.

Dabas et al. (1982) in their biochemical studies on 4 Red Dane and 4 Murrah bulls seminal plasma found that the values for both transaminases were non-significantly higher in Red Dane than Murrah bull seminal plasma. The GOT and GPT activity averaged 572.86 ± 37.41 and 99.64 ± 17.67 units/ml in Red Dane and 502.18 ± 68.53 and 88.25 ± 11.56 units/ml in Murrah bull seminal plasma, respectively.

Mohan and Razdan (1982) studied GOT and GPT activity during preservation of extended buffalo semen in 3 diluents at 5-6ºC up to 120 hrs. Initial GOT and GPT activity averaged 78.08 and 4.18, 67.97 and 3.74; 91.31 and 4.80 u mole/hr/ml in milk, whey and yolk diluents respectively. The differences being significant for both the activities.

Pandit and Garg (1983) studied freezability, GOT release and fertility of six cross-bred bulls’ semen using egg yolk citrates dilutor. The differences in GOT released between stages of freezing and between different breed crosses were significant. Post-thaw GOT release was higher (1043.64 units) in Brown Swiss crosses with low conception rates (37.5%) than in Jersey and Holstein crosses (976.61 and 979.07 units) with normal conception rates (53.33 and 48.57 % respectively), indicating negative relationship between GOT release and fertility.
Yaqub *et al.* (1983) studied level of transaminases and their relationship with physical characters of 4 Nill-Ravi buffalo bull semen. GOT and GPT activity in seminal plasma averaged 159.79± 8.48 and 15.59±0.69 µu/ml respectively.

Dhami (1986) studied the GOT (µmol/lit) and GPT (µmol/lit) levels in the seminal plasma of Surti bulls in different seasons (Hot, Wet and Cold). The average GOT values were 55.96±3.15, 41.29±2.29 and 57.26±1.87 µmol/lit respectively in three seasons and the GPT values were 12.26±1.47, 19.18±1.70 and 12.10±1.60 µmol/lit respectively for three seasons. The overall GOT and GPT levels in seminal plasma before freezing was 20.88±0.81 and 7.05±0.32 µmol/lit., which increased to 33.07±1.14 and 11.44±0.47 µmol/lit after post freeze.

Tuli *et al.* (1986) studied 40 ejaculates from 4 Murrah bulls diluted in Tris-glycerol with addition of raffinose, the GOT release in extra-cellular fluid in control group (TYG dilutor) was 89.00±11 unit/10⁹ sperm in pre freeze semen whereas 121.2±9.4 unit/10⁹ sperm after thawing.

Mustafa *et al.* (1987) conducted a trial in male Nubian goats. They studied semen morphology and biochemistry in furazolidone treated bucks. They observed that alkaline phosphatase activity was lower with 10 mg/kg of furazolidone and increased when the larger dose was administered.

Dhami and Kodagali (1988) reported that the mean values for GOT and GPT in Surti buffalo bulls semen were 51.50±1.60 µmol/lit and 14.51±0.97 µmol/lit respectively.

Dhami *et al* (1990) compared enzyme leakage in TFGG dilutor. The average GOT release in grade I and II semen ejaculates (according to motility) in Surti buffalo bulls was 19.26±1.40 µ mol/L and 23.39±2.49 respectively at pre freeze stage, where as 30.68±2.08 and 31.29±3.33 µ mol/L respectively at post thaw. The average GPT release was 6.93±0.66 and 6.84±0.89 µ mol/L respectively at pre freeze stage and 10.60±0.87 and 10.88±1.27 µ mol/L respectively at post thaw stage.

Singh *et al.* (1991) studied total 40 ejaculates from 5 Murrah buffalo bulls at weekly interval. The overall GOT (n mol pyruvate formed/min/ml ) in Tris yolk glycerol
after complete extension, after equilibration, 24 hrs post freezing and 7 days post freezing were 595.6±18.78, 610.2±34.89, 888.7±24.99 and 1250.8±68.11 respectively whereas GPT leakage was 69.20±4.58, 79.68±5.23, 101.22±8.89 and 138.64±10.91 respectively.

Tuli and Holtz (1992) studied twenty semen samples of Boer bucks by dividing into four equal parts and extending them at 37 °C in Tris, Test, Tes and Bes buffers containing egg yolk and glycerol. Semen were placed into medium size French straws and after 2 hours of equilibration at 5 °C, frozen in the vapour phase and stored in liquid nitrogen for 7 days at -196 °C. Glutamic oxaloacetic transaminase (GOT) release was studied after the initial extension, after equilibration and after 15 minutes and 7 days of freezing of semen. They recorded following concentration of GOT (Unit/ml) in different dilutors.

<table>
<thead>
<tr>
<th>Dilutors</th>
<th>Initial extension</th>
<th>After equilibrium</th>
<th>After 15 min of freezing</th>
<th>After 7 days of freezing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris egg yolk</td>
<td>83.30±5.30</td>
<td>92.15±4.60</td>
<td>128.75±6.80</td>
<td>132.00±6.30</td>
</tr>
<tr>
<td>Test egg yolk</td>
<td>78.95±4.50</td>
<td>94.00±5.00</td>
<td>135.00±7.30</td>
<td>137.0±6.50</td>
</tr>
<tr>
<td>Tes egg yolk</td>
<td>82.30±3.76</td>
<td>95.60±3.43</td>
<td>132.45±6.92</td>
<td>137.60±6.40</td>
</tr>
<tr>
<td>Bes egg yolk</td>
<td>80.40±4.08</td>
<td>93.60±4.15</td>
<td>134.00±5.90</td>
<td>136.70±7.50</td>
</tr>
</tbody>
</table>

No significant difference was noted in levels of GOT before and after freezing.

Chauhan et al. (1994) recorded following enzyme activity at each step over that estimated in undiluted semen of Jamnapari bucks:

<table>
<thead>
<tr>
<th>Freezing step</th>
<th>GOT Units</th>
<th>GPT Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before dilution</td>
<td>11.05</td>
<td>76.9</td>
</tr>
<tr>
<td>Cooling (5°C)</td>
<td>21.96</td>
<td>105.6</td>
</tr>
<tr>
<td>Equilibration (3 hours)</td>
<td>36.35</td>
<td>142.0</td>
</tr>
<tr>
<td>Freezing</td>
<td>58.28</td>
<td>217.3</td>
</tr>
</tbody>
</table>

They opined that GOT and GPT leakage in frozen thawed semen had positive correlation with acrosomal damage.

Tuli and Holtz (1994) evaluated semen of Boer goat for glutamic oxaloacetic transaminase (GOT) release at initial extension, equilibration and after 15 minutes and 7 days of freezing of semen. They recorded 82.5±3.0 and 108.1± 1.1 units/l GPT release prior to freezing and after thawing of semen. They opined that change of extenders did not influence the release of GOT at various stages of freezing of semen.
2.4.2 Phosphatases (AKP/ACP)

Semen Phosphatases play an important role in dephosphorylation in sperm metabolism. Phosphatases activity in sperm in mainly due to acid and alkaline phosphatase enzymes of its plasma. Acid phosphatase activity is directly related with androgenic activity, prostate functioning and fertilization. Phosphatases in semen reflect the functional state of accessory sex glands and metabolic activity of spermatozoa and are helpful in differentiating the reproductive biology of bulls of different breeds/species.

Roy et al. (1960) in their study on composition of bovine semen, reported the mean acid and alkaline phosphatase activity to be 308 ± 44 and 252 ± 37 units/100 ml of semen in buffalo bull and 145 ± 11 and 184 ± 14 units/100 ml in cow bull semen, respectively. The Phosphatase levels were significantly higher in buffalo semen than the cattle semen.

Eapen et al. (1964) in their studies on relationship between some seminal constituents in buffalo semen observed that the mean values of ACP and AKP were 601 ± 34 and 1001 ± 43 KAU/100 ml of semen respectively in 50 ejaculates from 10 Murrah bulls. AKP levels were significantly higher than ACP.

Murdoch and white (1968) reported that if a particular enzyme is present in whole semen, it occurs in both spermatozoa and seminal plasma. They recorded 1560±250, 2920±380, 15500±2300, 3660±150 and 80±30 unit/ml AKP in ram, bull, dog, rabbit and human whole semen respectively. They also reported 270±50,520±30, 2400±340, 90±50 and 168000±30300 unit/ml ACP in ram, bull, dog, rabbit and human whole semen respectively.

Misra et al. (1969) studied biochemical attributes of Murrah buffalo semen collected at various intervals. The mean phosphatase activity (Bu/100 ml) in the four successive ejaculate of Murrah bulls collected at eight days interval averaged to 89.99 ± 28.63, 286.22, 271.04 and 255.86 Bu/100 ml for ACP and 288.00 ± 23.07 , 264.30, 238.30 and 257.90 Bu/100 ml for AKP, respectively. The values were 317.70 ± 34.65, 279.19 ± 18.60 and 295.98 ± 18.02 Bu/100 ml for ACP and 300.50 ± 26.07,
288.15 ± 22.24 and 285.32 ± 16.93 Bu/100 ml for AKP, when the collection intervals were 72, 48 and 24 hrs respectively.

Zvereva and Cuhrii (1971) reported that the activities of alkaline and acid Phosphatase in seminal plasma from 24 Russian Black Pied bulls were 33 to 133 and 98 to 308 units respectively.

Kaker and Arora (1973) studied semen phosphatases of young crossbred bulls as affected by post pubertal age. There was a gradual increase in the levels of acid and alkaline phosphatases over the collections made at fortnight interval from HF x Hariana and BS x Hariana bulls. The mean value of ACP and AKP observed were 125 to 116 and 46 to 166 Bu/100 ml respectively.

Nafornita et al. (1977) studied some physical and chemical values and Phosphatase activity in bull semen in 3 winter months viz Dec, Jan, Feb based on 94 ejaculates. The activity of alkaline Phosphatase averaged 212.4, 313.8 and 455.3 units/ml and that of acid Phosphatase 282.1, 302.0 and 553.6 units/ml, respectively, effect of months being highly significant for both enzymes.

Reddy and Raja (1980) studied seasonal vibrations in acid and alkaline phosphatases content in 3 Surti buffalo bulls semen collected at weekly interval over 12 months period. For 17, 22 and 10 pooled samples examined during summer, monsoon and winter seasons respectively, showed no significant variation in the content of phosphatase enzymes. The overall values for acid and alkaline phosphatase were 757.13 ± 47.43 and 632.32 ± 3.04 units/100 ml respectively.

Kumar et al. (1984) in their comparative study on phosphatases in 3 successive semen ejaculates from 4 Red-Dane, 3 Jersey and 4 Murrah bulls showed that the buffalo seminal plasma had significantly higher levels of both phosphatases than cow bulls. The alkaline and acid phosphatases activity in 3 successive ejaculates of Murrah bulls averaged 294.41 ± 10.19, 279.87 ± 9.85 and 270.91 ± 9.25 Bu/100 ml and 201.49 ± 6.10, 207.66 ± 8.81 and 193.08 ± 6.36 Bu/100 ml of seminal plasma respectively. An increase in acid phosphatase was followed by an increase in alkaline phosphatase level.
Dhanotiya and Srivastava (1985) obtained buffalo seminal plasma from four bulls by centrifuging the semen sample. The ACP and AKP values were 68.0 and 18.5 enzyme activity respectively.

Ibrahim et al. (1985) studied the physico-chemical characteristics of pre-ejaculate fraction, whole semen and seminal plasma of 6 buffalo bulls, they reported that the content of both acid and alkaline (3.4±1.3 and 2.8±0.6 u/ml) phosphatases in the pre-ejaculate fraction (drip) seems to be negligible when compared to the whole semen (225 ± 3.0 and 326 ± 2.2 u/ml) and seminal plasma (230.5 ± 1.5 and 331.2 ± 2.6 u/ml). The differences between first and second ejaculates were not apparent.

Dhami (1986) studied the AKP (KAU/100ml) and ACP (KAU/100ml) levels in Surti buffalo bull seminal plasma during different season (Hot, Wet and Cold). The average AKP values were 559.65±13.45, 603.77±16.67 and 607.59±13.81 KAU/100ml respectively in three seasons, the ACP values were 266.41±4.93, 234.31±7.47 and 256.24±11.76 KAU/100ml respectively in three seasons. The overall mean AKP and ACP enzyme activity in pre-freeze seminal plasma were 68.02±3.72 and 31.60±1.74 KAU/100ml, which increased at post freeze to 94.32±4.50 and 53.22±2.46 KAU/100ml, respectively.

Mustafa et al. (1987) conducted a trial in male Nubian goats. They studied semen morphology and biochemistry in furazolidone treated bucks. They observed that alkaline phosphatase activity was lower with 10 mg/kg of furazolidone and increased when the larger dose was administered.

Dhami and Kodagali (1988) reported that the mean values for AKP and ACP in Surti buffalo bull seminal plasma were 590.34±70 KAU/100ml and 252.32±5.06 KAU/100ml respectively.

Dhami et al. (1990) compared enzyme leakage in TFYG dilutor. The average AKP release in grade I and II semen ejaculates (according to motility) in Surti buffalo bull seminal plasma was 49.58±5.43 and 49.37±8.72 KAU/100ml respectively at pre-freeze stage, whereas 69.26±6.12 and 71.30±10.39 KAU/100ml respectively at post thaw. The average ACP release was 29.56±3.07 and 35.44±4.54 KAU/100ml.
respectively at pre freeze stage and 48.37±3.49 and 59.51±6.86 KAU/100ml respectively at post thaw stage.

Azawi et al. (1990) studied sixteen semen ejaculates from two HF bulls preserved at ambient temp. The mean AKP and ACP values at 0 hrs were 17.64±1.47 and 10.52±1.5 IU/10^9 sperm.

Singh et al. (1992) studied 40 ejaculates from five buffalo bulls, the mean AKP and ACP in TYG at equilibrium and 24hr after freezing were 30.92±2.14 and 2.27±0.09, 76.02±5.90 and 7.08±0.47 n.mol.phenol liberated/min/ml respectively.

Chauhan et al. (1994) recorded following percent increase in enzyme activity at each step over that estimated in undiluted semen of Jamnapari bucks.

<table>
<thead>
<tr>
<th>Freezing step</th>
<th>AKP</th>
<th>ACP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before dilution</td>
<td>36.2</td>
<td>33.0</td>
</tr>
<tr>
<td>Cooling (5°C)</td>
<td>77.1</td>
<td>72.3</td>
</tr>
<tr>
<td>Equilibration (3 hours)</td>
<td>116.1</td>
<td>108.0</td>
</tr>
<tr>
<td>Freezing</td>
<td>135.8</td>
<td>136.0</td>
</tr>
</tbody>
</table>

They opined that AKP and ACP leakage in frozen thawed semen had positive correlation with acrosomal damage.

Sirat et al. (1996) studied semen samples from 12 bucks by extending in ten different extenders containing glycerol, DMSO, glycerol + DMSO, and glycerol + lactose in varying concentrations as cryoprotective agents. The activities of alkaline phosphatase (AKP), aspartate aminotransferase (AST), alanine amino transferase (ALT) were studied in equilibrated (Pre-freeze) and frozen thawed (Post-freeze) semen samples. They reported that extra cellular activities of alkaline phosphatase, transaminases (AST and ALT), were significantly higher in extenders containing DMSO than lactose. Leakage of these enzymes was found to increase from the pre-freeze to the post-freeze stage.

Zakrzewska et al. (2002) recorded 64.9 ± 14.6 U/L Acid Phosphatase (ACP) concentrations in Suffolk and Laine breeds of Ram in Poland.
2.4.3 Dehydrogenase (LDH)

Murdoch and white (1968) reported 33579±3258, 7383±2972, 1505±131, 11082±1037, 2536±555 unit/ml LDH in ram, bull, dog, rabbit and human whole semen respectively. These values on deep freezing of semen were 4068 and 2604 U/ml for ram and bulls.

Varshney et al. (1978) studied dehydrogenase activities in whole semen of 6 Murrah buffalo bulls. The LDH activity averaged 271.7± 18.74 ug/ml of semen.

Dube et al. (1982) studied lactic dehydrogense activities in relation to semen quality in 7 Jersey and 2 Murrah buffalo bulls. The units of lactic dehydrogenase per ml of undiluted semen were 1162.69 and 731.06 in Jersey and Murrah bulls, respectively, the difference being significant.

Dabas et al. (1982) reported significantly higher values of lactic dehydrogenase in seminal plasma of Red Dane (1109.82 ± 100.59 ug/ml) than the Murrah (204.38 ± 23.55 ug/ml) buffalo bulls.

Dhami (1986) studied the LDH (IU/L) levels in semen of Surti buffalo bulls in different seasons (Hot, Wet and Cold). The average LDH values in the semen were 395.94±10.15, 400.51±10.04 and 364.69±16.85 IU/L respectively in three seasons for Surti bulls. The overall level of LDH in pre freeze seminal plasma was averaged 114.61±5.77 IU/L, which increased to 156.24±6.57 IU/L after freezing.

Dhami and Kodagali (1988) reported that the mean values for LDH in Surti buffalo bulls semen was 387.05±7.47 IU/L.

Dhami et al. (1990) compared enzyme leakage in TFYG dilutor. The average LDH release in grade I and II ejaculates (according to motility) in Surti buffalo bulls was 120.85±11.92 and 105.75±14.83 IU/L respectively at pre freeze stage, where as 165.42±14.50 and 138.25±20.91 IU/L respectively at post thaw stage.
Azawi et al. (1990) studied sixteen semen ejaculates from two HF bulls preserved at ambient temp. The mean LDH value at 0 hrs was 124.57±9.27 IU/10⁹ sperm.

Singh et al. (1991) studied LDH activity of seminal plasma in five healthy Murrah bulls. The overall mean level of LDH in seminal plasma in TYG at equilibration and 24h after freezing was 81.40±1.82 and 164.52±22.00 n mol pyruvate reduce/min/ml.

Sirat et al (1996) studied semen samples from 12 bucks. They extended the semen samples with 10 different extenders containing glycerol, DMSO, glycerol + DMSO, and glycerol + lactose in varying concentrations as cryoprotective agents. Lactic dehydrogenase (LDH) was assayed in equilibrated (Pre-freeze) and frozen thawed (Post-freeze) semen samples. They reported that extra cellular activity of lactic dehydrogenase was significantly higher in extenders containing DMSO than lactose. Leakage of this enzyme was found to increase from the pre-freeze to the post-freeze stage.

Zakrzewska et al. (2002) recorded 64.9 ± 14.6 U/L LDH in Suffolk and Laine breeds of Ram in Poland.

Zamiri and Heidari (2006) studied levels of enzymes in Rayini male goats of Kerman province in Iran. Lowest level of lactate dehydrogenase (LDH) in the seminal fluid was recorded in early September (2.2 U/ml) and the highest level in November (2.5 U/ml).

2.5 Conception rates with frozen buck semen.

Corteel et al. (1974) observed in 296 and 293 females inseminated with deep-frozen and fresh semen respectively, the kidding percentage was 37.2 and 52.3 %. When 40X10⁶ - 80X10⁶ motile spermatozoa were used. Kidding percentages decreased from 50.0 to 22.4 % as doe milk yield increased from 450-599 to 800-1150 kg/yr. This inverse relationship was not observed when the insemination dose was increased to 150X10⁶ - 200X10⁶ motile spermatozoa. In the breeding season, the
kidding percentage in does inseminated with 40X10^6, 80X10^6 or 120X10^6 motile thawed spermatozoa was 42.5, 54.0 and 65.2 % respectively.

Corteel et al (1980) achieved the fertility rates by using frozen semen of Alpine and Saanen goat for AI in different season as -in Sep.-Nov., Dec.-Feb., Mar.-May and June-Aug. was 57, 66, 48 and 58% respectively. (551, 452, 294 and 250 females).

Lawrenz (1986) inseminated 218 goats with thawed frozen semen by non-surgical intra-uterine insemination technique. Of the various groups inseminated the percentage Boer does kidding was 60.8% (46) and 70.9% (31), the percentage Angora does kidding was 62.3% (53), 61.8% (68) and 60.0% (20). A dose of 75 X 10^6 sperm/ml was found to be suitable for intra-uterine insemination of Angora does.

Chauhan and Anand (1990) obtained 81% fertility rate in a trial conducted with frozen semen from Jamnapari bucks.

Ritar et al. (1990) conducted the experiments, using a total of 1833 Cashmere does the pregnancy rate for cervical (Experiment 1) and laparoscopic (Experiments 2 and 3) insemination was 39.1%, 63.6% and 52.1% respectively. After laparoscopic insemination, results were similar for semen frozen as pellets and in straws.

Ritar and Ball (1993) got 33.3% and 56.8% fertility rates after cervical and laparoscopic insemination of frozen-thawed buck semen. They also observed that there was no effect of egg yolk on the fertility after cervical insemination.

Sinha et al. (1995) got 50 to 60.20 % fertility rates with Tris based extended semen in Black Bengal, Beetal, and their cross-breeds.

Roca et al. (1997) inseminated Murciano-Granadina does with unwashed or washed spermatozoa and obtained 73.5% (25/34, does pregnant) and 70.3% (26/37, does pregnant) conception rate. In second experiment they used unwashed spermatozoa collected during the breeding and non-breeding season diluted in Tris-egg yolk extender and stored at 5°C used for artificial insemination. The fertility rates were indistinguishable between semen collected and processed in the breeding (84/115, 75% does pregnant) and non-breeding season (78/104, 73% does pregnant).
Karatzas et al. (1997) conducted trials in 4109 does of a local Greek breed for synchronization of oestrus. They used bucks of Alpine, Saanen and Damascus breeds for studying the fertility of fresh and frozen-thawed semen during the non-breeding season (June to August). Artificial insemination was performed once (50 to 55 h after sponge withdrawal) or twice (36 and 60 h) after sponge withdrawals with fresh semen or frozen semen. The overall kidding rate with fresh semen (65.5%) was higher (P < 0.05) than that with frozen-thawed semen (53.4%). The overall kidding rate was significantly higher (P < 0.001) in the does inseminated twice with fresh or frozen-thawed semen (70.4 and 59.1%, respectively) than in those inseminated only once (48.9 and 44.9%), respectively.

Greyling and Nest (2000) recorded 74.2% pregnancy rate following Artificial Insemination in Boer and Indigenous goats.

Romano et al. (2000) got 73.7% fertility for the treatment does (sterile service prior to insemination) and 58.7% control does.

Gacitua and Arav (2005) in an experiment achieved 58, 67, 50% pregnancy rates with fresh semen, whereas pregnancy rates with frozen semen were 33%, 37% and 53%. In the second experiment with pooled, washed semen they achieved 41.6%, pregnancy rate.

Paulenz et al. (2005) studied the effect of vaginal and cervical deposition of liquid semen stored at room temperature on the fertility of goats. In a field trial on 217 Norwegian dairy goats aged between 6 months and 7.5 years were inseminated after natural oestrous. Cervical insemination with $200 \times 10^6$ spermatozoa resulted 87.0% and 78.0% kidding rates, vaginal insemination gave 85.5 and 74.3% kidding rates. They observed no significant difference between the cervical and vaginal inseminations. They also observed that there were no significant differences between the fertility rates for different bucks. Fertility results after vaginal insemination were encouragingly high. Vaginal insemination is a simple, less costly and less time consuming technique compared to other methods.
Salvador et al. (2005) in artificial insemination programme studied the effect of factors such as depth of semen deposition, inseminator skill, Farm, sire and expression of oestrous on pregnancy rate in Murciano–Granadina (MG) goats during non-breeding season and using frozen semen. Frozen-thawed semen from six males was applied by three technicians to inseminate a total of 551 goats in 17 Farms distributed throughout the Mediterranean area of Spain. Pregnancy rate was determined at 6 weeks after insemination by trans-abdominal sonography. Overall pregnancy rate was 57%. They observed that deeper the semen was deposited in the genital tract, the higher was the rate of pregnancy obtained, being greater when the catheter reached the uterus.

Sohnrey and Holtz (2005) developed technique for trans-cervical deep uterine insemination of goats. They have compared trans-cervical method with the laparoscopic intrauterine insemination using frozen-thawed semen. They had achieved 71% fertility rate with trans-cervical insemination as compared with 53% in laparoscopic insemination.

Khalifa and Saidy (2006) obtained 55.26% kidding rates in natural and 53.85% in synchronized oestrus in does using frozen semen for artificial insemination.

Salvador et al. (2006) observed 47% versus 41% fertility rates with Artificial Insemination in does using buck semen stored in solid phase media and milk based extenders.

Mara et al. (2007) conducted a study in which goats were inseminated with diluted semen using different combination of extenders. The percentages of pregnant goats were 71.4%, 61.4% and 48.8% in different experiments. The kidding rates were 66.7% 61.4%, 48.8% in same experiments respectively.

Menchaca and Rubianes (2007) have got 40.3% to 63.7% pregnancy rates in different trials using frozen semen for timed AI.

Dogan et al. (2008) performed cervical artificial insemination with frozen-thawed semen once 16 h after the detection of the first accepted mount in does (n=80). The total pregnancy rate was found to be 33.3%.
Leboeuf et al. (2008) inseminated dairy goats out of the breeding season with deep frozen semen, after induction of oestrus and ovulation by hormonal treatments. They got 65% kidding rate to this treatment.

Batista et al. (2009) got 38.5% to 43.6% pregnancy rate in 150 Majorera goats by trans-cervical insemination. Goats were inseminated with frozen-thawed semen.

El-Kon et al. (2010) recorded 60% conception rate by using frozen thawed semen of Damascus bucks.
3. MATERIALS AND METHODS

The present study was carried out at the Instructional Livestock Farm Complex, Bombay Veterinary College, Unit Number 3, Aarey Colony, Goregaon (East), Mumbai-400065, Department of Animal Reproduction Gynaecology and Obstetrics, as well as at the National Institute for Research in Reproductive Health (NIRRH), Parel, Mumbai -400012. Six Osmanabadi and Six Sirohi bucks were selected on the basis of body condition, age and sexual behaviour for the study. The work was carried out for a period of 14 months from December 2008 to April, 2010.

3.1 Selection of animals

For the present research work twelve (6 Osmanabadi and 6 Sirohi) mature, healthy bucks of age between three to four years were selected from Instructional Livestock Farm Complex, Bombay Veterinary College, Arey Colony, Goregaon (East), Mumbai-65. All the bucks were maintained on ideal nutritional and managerial condition throughout the experimental period.

3.2 Sterilization of equipment and rubber material

All the glassware used during the study were thoroughly cleaned, dried and sterilized in hot air oven at 180° C for 1 hour. All the rubber material including Artificial Vagina and buffer solution were autoclaved at 15 lbs pressure (115° C) for 20 minutes.
Figure 1 Selected bucks

3.3 Semen collection
Semen was collected aseptically and hygienically, by Artificial Vagina method and it was maintained in 37˚C in water bath for further evaluation. Semen was collected from each buck at weekly interval. Three collections from each buck were obtained for analysis.

3.4 Statistical analysis

Data obtained for various parameters were subjected to statistical analysis as per Snedecor and Cochran (1970). Results are presented as Means ± S.E.

3.5 Semen evaluation of Osmanabadi and Sirohi bucks

Semen samples were evaluated for various macroscopic, microscopic and sperm function tests. The former consisted of volume, colour, consistency density, and pH while later consisted of mass activity, initial motility, total sperm count, live sperm percentage and abnormal sperm percentage. Additionally sperm function tests were performed to evaluate functional ability of sperm, which included Plasma Membrane integrity test (HOST), Acrosome Integrity (Geimsa staining), DNA integrity test (Acridine Orange staining and Comet assay) and Enzyme leakage (GOT/GPT, AKP/ACP and LDH) pre and post freezing (24 and 72 hours) stage.
3.5.1 Macroscopic evaluation of semen

Macroscopic evaluation of semen was carried by measuring volume, colour, consistency, density and pH as per the standard procedures.

3.5.1.1 Ejaculate volume

The volume and colour were evaluated immediately after collection, directly from the graduated semen collection cup.

3.5.1.2 Colour and Consistency

Colour was graded as creamy, milky, cloudy and watery, and consistency was recorded as thick and thin as per standard procedures based on fluid content of semen and sperm concentration.

3.5.1.3 Density

Density of the semen ejaculate was evaluated by holding the semen collection cup against light and graded from D to DDD.
Sirohi buck for semen collection

Teaser buck

Sniffing

Mounting

Protrusion of penis and thrush

Dismounting

Fig 2. Semen collection from selected bucks
3.5.1.4 Resazurin reduction test

1. Resazurin solution was prepared by mixing 50 mg Resazurin (7-hydroxy-3H, phenoxzin-3, one-10-oxide) in 100ml of 0.9% normal saline. This resazurin solution was used for further study.
2. 100 µl buck semen was taken in a test tube and 250µl resazurin solution was added. This solution was layered with small quantity of mineral oil.
3. Test tube was kept in incubator set at 37˚C for colour change.
4. The time required for colour change from dark blue to violet, violet to pink, and pink to white was noted in minutes.

3.5.2 Microscopic evaluation of semen

Microscopic evaluation of semen was carried out by measuring mass activity, initial motility, sperm concentration, percentage of live spermatozoa and percentage of abnormal spermatozoa.

3.5.2.1 Mass activity

Mass activity of semen was recorded by placing a small drop of freshly collected neat semen on clean glass slide, examining without cover slip, under a low power magnification (10X) (Salisbury and Van Demark 1978).

3.5.2.2 Initial motility

Initial motility of semen was measured by covering the semen drop with a thin cover glass under a high power magnification (40X) maintained at 35-37˚C. The motility was recorded as percentage of progressively motile spermatozoa, from 0 to 100.
3.5.2.3 Sperm concentration per ml

Concentration of spermatozoa (million/ml) in the neat semen was determined by the haemocytometer method adopting WBC counting procedure. Diluting fluid was prepared as: Sodium Citrate dehydrate 3 gm, Eosin yellow 100mg, Formalin 0.5 ml, Distilled water 100 ml.

3.5.2.4 Percentage of live spermatozoa

Percentage of live spermatozoa was estimated by differential staining technique using Eosin-Nigrosin stain (Campbell et al. 1952). The composition of the stain included Eosin-yellow 1.67 gm and 10 gm of Nigrosin in 100 ml of 2.90 % Sodium citrate buffer. The stain was ripened and then used. The smears were prepared in duplicate after mixing a small drop of neat semen with four drops of stain on a clean grease free microscopic slide at 37°C. A thin smear was prepared. Total 333 spermatozoa were counted under the objective (100X) of an oil immersion microscope for estimating the percentage of live spermatozoa. The unstained spermatozoa were counted as live and pinkish eosinophilic or partially stained spermatozoa were classified as dead.

3.5.2.5 Percentage of abnormal spermatozoa

The semen smears used for live sperm counts were also utilized to determine the percentage of morphological abnormal spermatozoa as per standard procedures (Blom, 1950).

3.5.2.6 Hydrogen ion concentration (pH) of semen

pH of neat semen was recorded by using pH paper.
3.5.3 Sperm function tests

After routine semen examination by macroscopic and microscopic tests, samples were subjected to additional sperm function tests to evaluate functional ability of the sperms by using Hypo-osmotic Swelling test (HOST), Acrosome Intactness test, Acridine orange test, and Comet Assay.

3.5.3.1 Hypo-osmotic Swelling test (HOS test)

1. Two hypo-osmotic solutions were prepared as follows-
   2.7% aqueous solution of fructose (1.351 gm/50 ml distilled water)
   1.47% aqueous solution of sodium citrate (0.735 gm/50 ml distilled water).
2. Equal volumes of both solutions (0.5 ml each) were mixed and kept in an incubator at 37˚C for 10 mins.
3. 50 µl of semen was added in above hypo-osmotic solution (fructose + sodium citrate) and incubated at 37˚C for 30 minutes
4. 10 µl of this mixture was taken on glass slide and covered with glass cover
5. Slide was observed under 10 X objective lens to determine the number of spermatozoa showing swollen head and coiled tail indicating sperms with intact plasma membrane (HOS positive sperm).
6. Total hundred spermatozoa were counted to determine the percentage of HOS positive spermatozoa.

3.5.3.2 Acrosomal intactness of spermatozoa

Acrosome damage was assessed by Giemsa staining as per Hancock (1951). A thin smear of semen was prepared on a clean glass slide. This smear was then air dried and fixed in 5% formaldehyde solution for 30 minutes at 37˚C temperatures. The slide was washed in distilled water and air dried. The acrosome staining solution was prepared by
thoroughly mixing; Giemsa stain 1 gm, Methanol 66 ml and Glycerol 60 ml and was then filtered. The filtrate was then used as a "stock solution".

"Working staining solution" was prepared by mixing 3 ml of stock solution with 2 ml Buffer saline solution at pH 6.8 and 45 ml carbon-di-oxide free double distilled water. The air dried slides of semen were kept in the staining jar containing working staining solution for 3 hours at 37\(^{0}\) C. The stained slides were washed with distilled water and air dried. Hundred spermatozoa were counted from each slide under oil immersion lens and spermatozoa with intact acrosome were noted to calculate the percentage of acrosomally intact spermatozoa.

3.5.3.3 Test for DNA damage

Assessment of DNA damage was done by two test
i) Acridine orange test
ii) Comet assay test

i) Acridine orange test

Acridine orange was performed as per protocol of Tejada et al. (1984).

Reagent preparation
1. Stock solution
   Acridine orange (AO) (from Hi-Media)  0.1 g
   Distilled water  100 ml
   Stored in dark

2. Working solution
   Stock solution  10 ml
   0.1 M Citric acid  40 ml
   0.3 M Na\(_2\)H PO\(_4\), 7 H\(_2\)O  2.5 ml

3. 0.1 M Citric acid
   Citric acid  5.234 g
   Distilled water  250 ml

4. 0.3 M Disodium Hydrogen Phosphate
Disodium Hydrogen Phosphate 4.06 g
Distilled water 100 ml

Procedure

a. A thin smear of semen was prepared.
b. Smear was fixed in Carnoy’s solution (3:1 methanol - glacial acetic acid) overnight
c. The slide was dried for a few minutes and working solution was layered (2-3 ml) over the slide and kept for 5 minutes.
d. The slide was rinsed in water and covered with cover slip.
e. The slide was read on a fluorescence microscope using 490 nm excitation filter and 530 nm barrier filter.
f. A total of 100 sperms were counted (red and green) twice and average was calculated. Green sperms indicated sperms with intact (non-damaged) DNA and red sperm indicated sperm with damaged DNA.

II) Single cell gel electrophoresis (Comet assay)

It was carried out as per procedure described by Nandre (2007) and Singh et al. (2003).

Material for Comet Assay

1. Lysing Solution (Stock)

2.5 M NaCl 146.10 g
100 mM Na2- ED. 37.22 g
10 mM Tris-HCl (pH-10) 01.20 g
1.0 % Triton X- 100 10.00 ml
1.0 % Sodium lauryl sarcosine 10.00 ml
Distilled water 1000.00 ml

Working lysing solution was made fresh each time by adding 0.5 mg/ml of Proteinase K to a warm stock of Lysing Solution (37°C).

2. Electrophoresis solution

300 mM sodium acetate 24.60 g
100 mM Tris-HCl, pH 8.0 01.20 g
Distilled water 1000 ml

3. Neutralization Buffer

0.4 M Tris (pH 7.4) 04.80 g
Distilled water 1000 ml

4. Agarose (made 24 hour prior to use)

0.5% low-melting point agarose
0.75% normal-melting point agarose.

5. Staining Solution

40 µl of 20 µg/ml of Ethidium Bromide

Procedure

1. Diluted semen sample was centrifuged at 800G for 10 minutes at 4°C. Seminal plasma (supernatant) was removed and remaining sperm cells were washed with Ca²⁺ and Mg²⁺ free PBS to yield a concentration of 1 x 10⁷ spermatozoa/cm³.

2. Agarose slides were prepared as follows:
Base layer First Coat: 100 µl 0.75% normal melting point agarose was poured on clean frosted glass slides and covered with cover glass, and kept on icepack at 4°C. Cover glass was removed after two minutes, gently by sliding.

3. Five micro liters of cell suspension was taken and mixed with 75µl of 0.5% Low melting point agarose. It was then layered on pre-coated frosted slides, kept on an ice pack for 2 minutes and cover-slip was gently removed by sliding. The slides were kept protected from light until they were ready to be put in to lysing solution.

4. Working lysing solution was prepared fresh from stock by adding 0.5mg/ml of Proteinase K. Slides were dipped in the lysing solution and incubated in dark for 24 hours at 4°C.

5. Slides were equilibrated in an electrophoresis solution for 15 minutes before being electrophoresed at 12 V (0.46 V/cm, 100 mA) for 2 hours at room temperature.

6. The slides were drained, flooded slowly with neutralization buffer, fixed in 70 % ethanol for 15 min and then air-dried.

7. The dried slides were stained with 40 µl of 20 µg/ml Ethidium bromide.

8. Spermatozoa to be analyzed for comets were visualized immediately under an epifluorescence microscope. Whole sperm heads without a comet were considered as not damaged where as spermatozoa with fragmented DNA that migrated from the sperm head causing a "Comet" pattern were considered damaged.

9. A total of 100 sperm cells per slide were assessed for comets. The comets were captured with a still camera connected to the fluorescent microscope and the images were evaluated for percentage of tailed DNA.
3.5.4 Enzyme leakage test

3.5.4.1 Estimation of GOT and GPT in seminal plasma

Glutamic Oxalo-acetic Tranaminase (GOT) and Glutamic Pyruvic Transaminase (GPT) were estimated in, neat semen, pre-freeze and post-freeze seminal plasma on an autoanalyser.

a) Glutamic Oxalo-acetic Tranaminase (GOT)

For estimation of GOT leakage, reagent kit used was of Diachem Ltd. In reagent kit, regent 1(R1) contains L-Aspartate and reagent 2 (R2) contains α-Ketoglutarate.

Procedure

700µl R1 and 350µl R2 were mixed in cuvett tube. In this mixture 100µl seminal plasma was added. Tube was kept in auto analyzer (Stat Fax 2000) for further reading. Which was noted down?

b) Glutamic Pyruvic Transaminase (GPT)

For estimation of GPT leakage, reagent kit used was of Diachem Ltd. In reagent kit, regent 1(R1) contains L-Alanine and reagent 2 (R2) contains α-Ketoglutarate.

Procedure

800µl R1 and 200µl R2 were mixed in cuvett tube. In this mixture 50µl seminal plasma was added. Tube was kept in auto analyzer (Stat Fax 2000) for further reading which was noted down
3.5.4.2 Estimation of AKP and ACP in seminal plasma

Alkaline phosphate (AKP) and Acid phosphate (ACP) in neat semen, pre-freeze and post-freeze seminal plasma were determined by using on an auto analyzer (Stat-Fax 2000).

a) **Alkaline phosphate (AKP)**

For estimation of AKP leakage, reagent kit used was of Aspea Laboratory. In reagent kit, diluents (R1) contains Diethanolamine buffer and substrate (R2) contains p-Nitrophenyl phosphate Sodium Chloride.

**Procedure**

Powder R1 0.1 g and 1000 µl R2 were mixed in cuvett tube. In this mixture 20µl seminal plasma was added. Tube was kept in serum analyzer (Stat Fax 2000) for further reading.

b) **Acid phosphate (ACP)**

For estimation of ACP leakage, reagent kit used was of Aspea Laboratory. In reagent kit, ACP reagent 1(R1) contains α-naphthylphosphate and reagent 2 (R2) contains distill water.

**Procedure**

ACP reagent (R1) 1000 µl and 1000 µl R2 were mixed in cuvett tube. In this mixture 100µl seminal plasma was added. Tube was kept in serum analyzer (Stat Fax 2000) for further reading.
3.5.4.3 Estimation of LDH in seminal plasma

Lactate Dehydrogenase (LDH) was measured in neat semen, pre-freeze and post-freeze seminal plasma on an auto analyzer. For estimation of LDH leakage, reagent kit used of Diachem Ltd.

In reagent kit, reagent 1(R1) contains sodium pyruvate and reagent 2 (R2) contain NADH.

Procedure

500 µl reagent (R1) and 500 µl R2 were mixed in cuvett tube. In this mixture 25 µl seminal plasma was added. Tube was kept in serum analyzer (Stat Fax 2000) for further reading.
3.6 Dilution of semen

3.6.1 Dilutors used for semen preservation

The dilutors used for preservation of semen were Tris Egg yolk Glycerol (TYG) dilutor (Davis et al., 1963), Tris Egg yolk Ethylene Glycol (TYE) dilutor (Rohilla et al., 2005) and Tris -Dimethyl sulphonyl oxide (DMSO) dilutor (TYD).

Preparation of dilutors

1. Tris Buffer
   Tris (Hydroxymethyl amino methane): 30.48 g
   Citric Acid monohydrate: 17.00 g
   Fructose (anhydrous): 12.50 g
   Double glass distilled water up to: to make volume 1000 ml

2. Tris egg yolk glycerol (TYG) dilutor:
   Tris buffer: 90 ml
   Fresh egg yolk: 05 ml
   Glycerol: 05 ml

3. Tris egg yolk ethylene glycol (TYEG) dilutor:
   Tris buffer: 90 ml
   Fresh egg yolk: 05 ml
   Ethylene glycol: 05 ml

4. Tris egg Dimethyl Sulphoxide (DMSO) dilutor
   Tris buffer: 90 ml
   Fresh egg yolk: 05 ml
   Dimethyl Sulphoxide (DMSO): 05 ml

Dilutors were prepared by standard procedure.
Semen dilution

Diluted semen

French medium straws for different diluter

Straw filling

Straw sealing

Straw preservation

Figure 3 Semen preservation
3.6.2 Dilution rate and freezing of semen

Immediately after collection of semen 20 µl of neat semen was taken in a glass test tube for evaluation. Then in remaining part of semen 2 ml of Tris egg yolk buffer was added and kept in refrigerator for cooling. Diluted semen was divided equally in three test tubes and different cryo-protectants were added. This diluted semen was taken to frozen semen laboratory and kept at 5°C for two hours for equilibration. From each test tube 200 µl of cooled semen was taken for evaluation. Dilution was done to have 160 to 180 millions sperm /ml.

3.7 Preservation of semen at sub-zero temperature

3.7.1 Printing of french medium straws

The French medium straws (0.5 ml) were printed by automatic printer machine for identification of buck number, breed, dilutor used and date.

3.7.2 Filling and sealing of french medium straws

Previously diluted semen which was kept for equilibration was filled in French medium straws (0.5ml) and laboratory plug was applied using polyvinyl powder after introducing little air using bubbler to prevent breakage of straws during deep freezing protocol.
3.7.3 Freezing of straws

The equilibrated straws were placed on the racks which were placed on grill present in Liquid Nitrogen container. Level of liquid nitrogen (LN₂) was kept up to grill, 20 minutes before freezing. The straws were frozen 4 cms above LN₂ level for 10 minutes to achieve temperature of -140°C. Thus, the average freezing rate was -14.5°C per minute. The straws on each rack were collected and quickly immersed in respective goblets containing liquid nitrogen and these goblets were transferred to liquid nitrogen container.

3.8 Evaluation of thawed semen

The post thaw evaluation progressive motile sperm percentage, live sperm percentage and abnormal sperm percentage, hypo-osmotic swelling test (HOST) was carried out at Instructional Livestock Farm Complex, Bombay Veterinary College, Acrosome intact test, Acridine orange test (AOT) and single cell gel electrophoresis (Comet assay) were carried out at National Institute of Research in Reproductive Health (NIRRH), Parel, Mumbai. Enzyme leakage (GOT, GPT, AKP, ACP and LDH) tests were also carried out at Instructional Livestock Farm Complex, Bombay Veterinary College on auto analyzer (Stat Fax 2000 machine).
3.9 Study of conception rate

3.9.1 Thawing of semen

The French medium straws were removed from LN₂ container and thawed in water bath at 37°C for 30 seconds just before artificial insemination.

3.9.2 Artificial Insemination.

Conception rates were studied in Osmanabadi goats by using frozen semen inseminations of Osmanabadi, Sirohi, Boer and Damascus bucks.

The Osmanabadi goats were synchronized for oestrous by using vaginal sponge of progesterone procured from Central Sheep and Wool Research Institute (ICSWRI) Avikanagar. Before oestrous synchronisation the goats were scanned by ultra sonography for pregnancy twice at 15 days apart. Than non pregnant goats were selected for the oestrous synchronization. The progesterone sponge was inserted in the vaginal passage of goats and it was kept there for 14 days. On day 14 the sponge was removed and injection PMSG 100 IU was given to each goat intra muscularly. The goats were inseminated at 24 and 48 hours after the injection of PMSG.

Six Osmanabadi goats were inseminated by using frozen semen from each buck of Osmanabadi and Sirohi breed frozen in three different dilutors. (Total 216 goats were inseminated).

Twelve Osmanabadi goats were inseminated by using frozen semen from one each buck of Boer and Damascus breed (Total 24 goats were inseminated).

Vaginal Artificial Insemination was carried out in oestrus synchronized goats at Instructional Livestock Farm Complex, Goregaon and Poonya Shlok Ahilya Devi sheep and goat Farm, Tirth (Budruk), District Tuljapur, Maharashtra.

Previously frozen semen from selected bucks was thawed following above mentioned procedure. In selected goats Artificial Insemination was done using frozen thawed semen. At the time of insemination hind limbs of goat were lifted from behind, then cervix was located with the help of vaginal speculum, after that semen was deposited by
Vaginal progesterone sponges

Sponge insertion in Osmanbadi goat

Heat detection using apron bucks

Artificial Insemination

Ultra sonography of inseminated goats for pregnancy diagnosis

Figure 4 Oestrous synchronization and Artificial Insemination in does
passing one fold of cervix or at the external os- cervix with the help of French insemination gun. Goat number and details of straws used were recorded.

3.9.3 Pregnancy diagnosis

Pregnancy diagnosis of inseminated goats was done between 27 and 30 days after insemination by ultrasonography using trans- abdominal probe.
4. RESULTS AND DISCUSSION

The present research work was carried out at the Instructional Livestock Farm Complex, National Institute for Research in Reproductive Health (NIRRH), Parel, Mumbai-12, Department of Animal Reproduction Gynecology and Obstetrics, Bombay Veterinary College and Frozen Semen laboratory, Kokan Development Corporation Ltd, Aarey Colony, Unit No.16, Goregaon (East), Mumbai-400 065. Total 12 bucks (six Osmanabadi and six Sirohi) belonging to Instructional Livestock Farm Complex were used for the research work. Semen evaluation was carried at laboratory of Livestock Farm and NIRRH. Semen freezing and preservation was done at frozen semen laboratory, Kokan Development Corporation Ltd. Artificial Insemination trials were performed on goats of Instructional Livestock Farm Complex and Punya Shlok Ahilyadevi Sheep and Goat Farm, Tirth (Budruk), Taluka Tuljapur, Dist Solapur, Maharashtra state.

Prior to start of present research work complete physical examination of all selected bucks was done. With andrological examination, disorders and abnormalities in testicles, epididymis, prepuce, penis, scrotum and spermatic cords, were thoroughly checked. Their libido and semen picture was also studied. Thus all the selected bucks for the present study were having normal reproductive organs, normal libido and normal semen picture. All the bucks were physically normal and healthy.

Semen evaluation

Semen samples of 12 bucks (6 Osmanabadi and 6 Sirohi) were evaluated for various macroscopic and microscopic tests. Three samples were collected in all bucks at an interval of 7-15 days period. The repeat sample was treated as a new sample and the observations for seminal attributes were pooled for statistical analysis. Semen was collected in clean graduated glass collection cups by artificial vagina method. After collection volume, colour, consistency, density were evaluated by visual
observation. pH was noted by using pH paper. Semen was kept in a water bath at 37˚C, till further evaluation was carried out.

Seminal attributes of neat semen of Osmanabadi and Sirohi bucks are given in table no 1

Table 1 Seminal attributes of neat semen of Osmanabadi and Sirohi bucks.

<table>
<thead>
<tr>
<th>Seminal attributes</th>
<th>Osmanabadi</th>
<th>Sirohi</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macroscopic tests</strong></td>
<td>Mean ± S.E n=18</td>
<td>Mean ± S.E n=18</td>
<td></td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>1.7 ± 0.11</td>
<td>1.8 ± 0.07</td>
<td>0.430</td>
</tr>
<tr>
<td>Colour</td>
<td>Yellowish to Yellowish white</td>
<td>Yellowish white to Creamy white</td>
<td></td>
</tr>
<tr>
<td>Consistency</td>
<td>Creamy to Thick creamy</td>
<td>Creamy to Thick creamy</td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>DDD(D) to DDDD</td>
<td>DDD(D) to DDDD</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>6.70±0.03</td>
<td>6.65±0.02</td>
<td>1.349</td>
</tr>
<tr>
<td><strong>Microscopic tests</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mass activity</td>
<td>4.00 ±0.00</td>
<td>4.00 ±0.00</td>
<td>1.000</td>
</tr>
<tr>
<td>Sperm concentration (millions/ml)</td>
<td>2616.8 ± 39.12</td>
<td>2364.7 ± 75.50</td>
<td>2.803*</td>
</tr>
<tr>
<td>Initial sperm motility %</td>
<td>86.66 ± 1.72</td>
<td>86.13 ± 1.33</td>
<td>0.297</td>
</tr>
<tr>
<td>Live sperm %</td>
<td>86.05 ± 0.41</td>
<td>84.05 ± 0.79</td>
<td>2.130*</td>
</tr>
<tr>
<td>Abnormal sperm %</td>
<td>13.28 ± 1.09</td>
<td>11.78 ± 0.90</td>
<td>1.548</td>
</tr>
<tr>
<td><strong>Sperm function tests</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma membrane integrity (Hypo-Osmotic Swelling Test- HOST) %</td>
<td>83.13 ± 1.18</td>
<td>78.33 ± 1.78</td>
<td>2.294*</td>
</tr>
<tr>
<td>Acrosome Intact sperm %</td>
<td>91.94 ± 0.75</td>
<td>90.94 ± 1.24</td>
<td>0.689</td>
</tr>
<tr>
<td>DNA damaged sperm % (Acridine Orange test)</td>
<td>00.00 ± 0.00</td>
<td>00.00 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>DNA damaged sperm % (Comet assay)</td>
<td>00.00 ± 0.00</td>
<td>00.00 ± 0.00</td>
<td></td>
</tr>
<tr>
<td><strong>Enzyme activity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamic- Oxaloacetic Transaminase (GOT) IU/L</td>
<td>82.55±6.95</td>
<td>73.61±4.37</td>
<td>1.434</td>
</tr>
<tr>
<td>Glutamic- Pyruvate Transaminase (GPT)IU/L</td>
<td>16.94 ±0.84</td>
<td>16.06±0.81</td>
<td>1.375</td>
</tr>
<tr>
<td>Alkaline Phosphates (AKP) (IU/L)</td>
<td>970.88±5.39</td>
<td>974.05±4.24</td>
<td>0.761</td>
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<tr>
<td>Acid Phosphates (ACP) IU/L</td>
<td>48.95±1.29</td>
<td>47.38±0.80</td>
<td>1.187</td>
</tr>
<tr>
<td>Lactate Dehydrogenase LDH (IU/L)</td>
<td>293.44±9.28</td>
<td>281.55±6.43</td>
<td>1.495</td>
</tr>
</tbody>
</table>

*Significant at 5% level
4.1 Macroscopic characteristics of neat semen of Osmanabadi and Sirohi bucks

4.1.1 Ejaculate volume

From table 1 it is revealed that the average ejaculate volume for Osmanabadi and Sirohi bucks was 1.7±0.11 ml and 1.8±0.07 ml respectively. There was no significant difference in the ejaculate volume between two breeds.

The volume of ejaculate recorded in present study for Osmanabadi breed was in the range of 0.9 to 2.0 ml and that of Sirohi bucks was 1.2 to 2.2 ml. The volume of ejaculate recorded in Osmanabadi bucks in the range of 0.56 to 1.15 ml by Baviskar (1985), Parandekar (1987), Puranik (1988) and Bakshi (1991) was found to be lower than present observations. This difference may be attributed to the plane of nutrition and climatic variations.

Austin et al. (1968) recorded 1.93 ml volume of semen in Fawn white German bucks which was in close agreement with present findings.

The volume of semen recorded in the range of 0.38 to 1.4ml in different breed of bucks by following authors was found to be lower than present observations. Mockel (1937) in Fawn White German breed, Nimkar (1977) in Surti bucks, Ahmad and Noakes (1996) in young British bucks, Prado et al. (2002) in Mexican bucks, Silvestre et al. (2004) in young Murciano-Granadina bucks, Zamiri and Heidari (2006) in Iranian Rayini goats. Dorado et al. (2009) in mature Florida bucks, Berlinguer et al. (2009) in Italian bucks and El-Kon et al. (2010) in Damascus bucks. The volume of semen recorded in nondescript bucks in the range of 0.72 to 0.86 ml by Keshavreddy (1983); Kishore and Rao (1983) was also lower than present observations. This difference may be attributed to the breeds of bucks, age, climatic variation, testicle size of bucks and managmental changes.

The volume of semen recorded in Angora bucks in the range of 0.8 to 1.09 ml by Cetinkaya et al. (1980), Dunder et al. (1983), Loubser et al. (1983), Serving et al. (1985), Bakshi et al. (1987) and Mendoza et al. (1989) was found to be slightly lower than present observations.
Figure 5 Semen ejaculate
4.1.2 Colour, Consistency and Density of neat semen.

From the table 1 it is evident that colour of semen varied from yellowish to yellowish white in Osmanabadi bucks, where as it varied from yellowish white to creamy white in Sirohi bucks.

The colour, consistency and density of neat semen from Osmanabadi or Osmanabadi crossbred buck recorded by, Baviskar (1985) Parandekar (1987) Puranik (1988) and Bakshi (1991) are in agreement with the observations recorded in present study.

The colour and consistency of neat semen of bucks recorded by Mockel (1937) in German white breed, Nimkar (1977) in Surti bucks, Pandey et al. (1985) in Saanen and Barbari bucks, Bakshi et al. (1987) and Mendoza et al. (1989) in Angora bucks and Ahmad and Noakes (1996) in young British bucks are also in agreement with the observations recorded in present study.

4.1.3 Hydrogen ion concentration (pH) of semen

From table 1 it is clear that the average pH of semen of Osmanabadi and Sirohi bucks was 6.70±0.03 and 6.65±0.02 respectively. There was no significant difference between two breed with respect to pH of neat semen.

The pH of semen ejaculate recorded in present study for Osmanabadi bucks was in the range of 6.5 to 6.9 and that of Sirohi bucks was 6.5 to 6.8. Similar observation was recorded by Parandekar (1987) and Bakshi (1991) in Osmanabadi bucks, Knoblauch (1962) in German white bucks, Koh (1975) in Katjang x Jamnapari bucks, Prasad et al. (1986) in Sannen x Barbari bucks and Greylinga and Grobbelaar (1983) in Boer and Angora bucks.

The difference in the values of pH of neat semen may be attributed to variation in breeds, age, plane of nutrition, climatic change and management variations of bucks.

4.1.4 Resazurin test

The average time required for changing colour in resazurin test of neat semen of Osmanabadi and Sirohi buck semen is given in table 2.

<table>
<thead>
<tr>
<th>Breed</th>
<th>Dark blue to violet</th>
<th>Violet to Pink</th>
<th>Pink to white</th>
<th>Total time Blue to white</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmanabadi</td>
<td>1.32±0.08</td>
<td>4.14±0.21</td>
<td>10.50±0.40</td>
<td>15.96±0.66</td>
</tr>
<tr>
<td>Sirohi</td>
<td>2.24±0.13</td>
<td>3.95±0.06</td>
<td>13.20±0.69</td>
<td>19.38±0.13</td>
</tr>
</tbody>
</table>

There was no significant difference (P>0.05) between two breeds of bucks with respect to resazurin test.

The literature pertaining to resazurin reduction test for bucks semen was not available hence comparison with other species was made.

The average time required in present study for reduction of resazurin from blue to white (15 to 19 minutes) was in close agreement with the observations of Martin et al. (1999) in Rambouillet rams, Pathak (1989) in crossbred bulls, Dart (1994) in Limousine bulls and Foote (1999) in crossbreed bulls.

Erb et al. (1952) recorded slightly higher time for resazuri reduction test (blue to pink) than present observations. This may be attributed to the sperm concentration in semen, sexual health of animals etc.
Figure 6 Resazurin test
4.2. Microscopic evaluation of semen of Osmanabadi and Sirohi bucks

The microscopic characteristics of neat semen of Osmanabadi and Sirohi bucks semen are given in table 1.

4.2.1 Mass activity

From table 1 it is clear that the average mass activity of Osmanabadi and Sirohi buck semen was in the grade of + 4.

There was no significant difference (P>0.05) between mass activities of two breeds of bucks.

Parandekar (1987), Puranik (1988) and Bakshi (1991) recorded slightly lower mass activity in Osmanabadi bucks ejaculates than that of present observations.

The mass activity of semen recorded by Nimkar (1977) in Surti bucks and Tuli and Holtz (1992) in Boer bucks was lower than present observation.

The mass activity of semen recorded in the range of 4.2 to 4.5 by Sinha and Singh (1982) in Black Bengal and Saanen bucks, Singh et al. (1985) in Jamnapari X Black Bengal bucks, Prasad et al. (1986) in Saanen X Barbari bucks was found to be slightly higher than the present findings.

The difference in mass activity of bucks semen could be attributed to seasonal variation. According to Ahmad and Noakes (1996) the mass motility of male goats semen at south England was higher during August to December than that in remainder of the year. The other contributory factors affecting mass activity of semen are plane of nutrition, libido etc.

4.2.2 Sperm concentration (millions/ml)

From table 1 it is seen that the sperm concentration of Osmanabadi and Sirohi buck neat semen was 2616.80 ± 39.12 and 2364.70± 75.50 million/ml respectively.
There was a significant difference (P<0.05) between two breeds with respect to sperm concentration. The sperm concentration was higher in case of Osmanabadi than Sirohi bucks.

Bakshi (1991) recorded 2924.64±24.17 millions/ml sperm concentration in semen from Osmanabadi bucks which was higher than present observations.

Puranik (1988) recorded similar type of sperm concentration in Osmanabadi crossbred bucks as present findings. However, Baviskar (1985) and Parandekar (1987) recorded higher sperm concentration in Osmanabadi crossbred bucks than present observations.

The sperm concentration in semen of different breeds of bucks recorded in the range of 2200 to 2800 millions/ml by following authors was in close agreement with present observations.


The sperm concentration in semen of different breeds of bucks recorded higher than 3000 millions/ ml by following authors was higher than present observations.


Berlinguer et al. (2009) recorded lower sperm concentration than present observation in bucks from Italy.
The variation in sperm concentration may be attributed to age of bucks, season of semen collection, frequency of semen collection, plane of nutrition and breed of bucks.

### 4.2.3. Initial sperm motility percent in neat semen.

From table 1 it is evident that the average initial sperm motility percentage of Osmanabadi and Sirohi bucks semen was 86.66 ±1.72 and 86.13± 1.33 respectively.

There was no significant difference (P>0.05) between two breeds of bucks with respect to initial sperm motility percent.


The initial sperm motility in semen of different breeds of bucks recorded in the range of 80 to 90 percent by following authors was also in close agreement with present observations.


The initial sperm motility in fresh semen of bucks of different breeds was recorded in the range of 70 to 80 percent by following authors which was found to be lower than present observations.


The initial sperm motility recorded by Dorado et al. (2009) in fresh semen of mature Florida bucks (2-year old) is comparatively higher than present findings.
The variations in initial sperm motility percentage in bucks semen may be attributed to difference in breed, season of semen collection, plane of nutrition of bucks etc.

4.2.4 Live sperm percentage in neat semen.

From table 1 it is clear that the live sperm percentage of Osmanabadi and Sirohi bucks semen was 85.83 ± 0.41 and 84.06 ± 0.79 respectively.

There was a significant difference (P<0.05) between two breeds of bucks with respect to live sperm percentage.

Significantly higher percentages of live spermatozoa was noted in neat semen of Osmanabadi than Sirohi bucks.

The observations of live sperm percentage in semen of Osmanabadi bucks in present study are in agreement with Baviskar (1985) for Osmanabadi bucks semen; but Parandekar (1987), Puranik (1988) and Bakshi (1991) reported lower live sperm percent in Osmanabadi buck semen than present observations.

The present observations pertaining to live sperm percentage are in close agreement with that of Austin et al. (1968) in Spanish bucks, Nimkar (1977) in Surti bucks, Bakshi et al. (1987) in Angora bucks and even in Jamnapari X Beetal cross, Sannen X Barbari cross and Angora cross reported by Singh et al. (1985), Prasad et al. (1986) and Bakshi et al. (1987) respectively.


Higher live sperm percentage than present observation was recorded by Chauhan et al. (1994) in semen of Jamnapari bucks and Oyeyemi et al (2001) in West African Dwarf bucks,
The variation in the live sperm percentage in semen may be attributed to difference in staining procedure, breeds, plane of nutrition and seasonal variations.

4.2.5 Abnormal sperm percentage in neat semen.

From table 1 it is seen that the abnormal sperm percentage in Osmanabadi and Sirohi bucks semen was 13.28 ± 1.09 and 11.78 ± 0.90 respectively.

There was no significant difference (P>0.05) between two breeds with respect to abnormal sperm percentage count.

Non significantly higher percentage of abnormal spermatozoa was observed in Osmanabadi bucks than Sirohi bucks.


The abnormal sperm percentage in semen of different breeds of bucks recorded in the range of 10 to 14 percent by Saxena and Tripathi (1980) in Jamnapari bucks, Keshavreddy et al. (1989) in local bucks ageing more than 2 years and Zamiri and Heidari (2006) in Rayini bucks was in close agreement with present observations. However lower abnormal sperm percentage in semen of different breeds of bucks was recorded in the range of 5 to 9 percent by Austin et al. (1968) in Spanish bucks, Nimkar (1977) in Surti bucks and Sinha and Singh (1982) in Saanen bucks. Higher values in the range of 19 to 23 percent were noted by Cetinkaya et al. (1980), Loubser et al. (1983) and Servinc et al... (1985) in Angora bucks.

As per Roca et al. (1992) and Ahmad and Noakes (1996) variations in sperm abnormality may be attributed to seasonal variation. The other factors that contribute to variation in sperm abnormality may be plane of nutrition and management of breeding bucks.

The permissible limit of abnormal sperm percentage in semen is less than 20 percent; hence semen samples from both the breed were within prescribed limits and
were fit for further processing. In brief it can be concluded that the quality of Osmanabadi and Sirohi buck semen (macroscopic and microscopic) was excellent. All the values were in the normal physiological ranges.

The variations recorded in the neat semen quality (macroscopic and microscopic) in present study in comparison with those reported by various authors may be because of breed variation, seasonal variation, age variation and variation in feeding and management conditions.
4.3 Evaluation of neat semen of Osmanabadi and Sirohi bucks using sperm function test

The sperm function test of neat semen of Osmanabadi and Sirohi bucks semen are given in table 1

4.3.1 Plasma membrane integrity (Hypo-Osmotic Swelling Test- HOST)

From table 1 it is evident that 83.13±1.18 and 78.33±1.78 percent spermatozoa had shown swelling under HOS test in Osmanabadi and Sirohi bucks neat semen respectively.

There was a significant difference (P<0.05) between two breeds of bucks with respect to HOS sperm percent. The Osmanabadi buck semen was showing significantly higher plasma membrane integrity in neat semen as compared to Sirohi bucks.

The literature pertaining to bucks semen particularly for HOST was scanty hence the comparison with other species was made.

The results of HOS sperm percentage recorded by Quintela et al. (2010) in canine and Li et al. (2005) in Cynomolgus Monkey are in agreement with present study.

The HOS sperm percentage recorded in the range of 28 to 64 percent by Fonseca et al. (2005) (51.5%) in Alpine and Sannen bucks, Nur et al. (2005) (53.7%) in Sannen bucks and Salvador et al. (2006) (28.0%) in Murciano-Granadina bucks were lower than present findings.

The HOS sperm percentage obtained in the range of 60 to 70 by Gang Zhang et al. (2007) in bucks from china (64.26%). Temwong et al. (2006) in swamp buffalo bulls and Gupta and Misra (2007) in buffalo bull (65%) were slightly lower than present observations.

The HOS sperm percent varies according to bulls (Prasad et al.1999), bucks, or season (Kale et al.2000).
4.3.2 Acrosome integrity (Giemsa stain)

From table 1 it is evident that the average acrosome intact sperm percentage of Osmanabadi and Sirohi bucks neat semen was 91.94±0.75 and 90.94±1.24 respectively.

There was no significant difference (P>0.05) between of two breeds of bucks with respect to Acrosome Intactness in neat semen.

The literature pertaining to bucks semen particularly for acrosome intactness was scanty hence the comparison with other species was also made.

The acrosome integrity recorded near 90 percent by Chauhan et al. (1994) in Jamnapari bucks, Li et al.(2005) in Cynomolgus Monkey, Dorado et al. (2009) in Florida bucks and El-Kon et al.(2010) in Damascus bucks semen was in agreement with present observations.

However, acrosome integrity recorded in the range of 60 to 80 percent by Salvador et al. (2006) in Murciano-Granadina bucks, Rafiquel Islam et al. (2006) in crossbred (Beetal × Assam Local) bucks and Gang Zhang et al. (2007) in bucks from china was lower than present observations. This may be due to difference in breed of bucks.
Figure 7 Sperm Acrosome Intactness test of neat semen (Giemsa stain)
Reyes et al. (2002) recorded 97.2 per cent acrosomal intactness in fresh boar semen which was higher than present observations. This may be due to species variation.

4.3.3 DNA damage

4.3.3.1 DNA damage (Acridine orange test)

From table 1 it is evident that no DNA damage detected in Osmanabadi and Sirohi bucks neat semen using Acridine Orange test.

The literature pertaining to bucks semen particularly for DNA damage in neat semen was not available; hence the comparison with other species was made.

Kosower et al. (1992) recorded only one percent DNA damage in human and no DNA damage in hamster neat semen which was in close agreement with present observations.

El-Nattat (2011) recorded 6% DNA damage in rabbits which was higher than present observations.

The DNA damage recorded in the range of 5 to 30 percent in human by Eggert-Kruz (1996), Spano et al. (1999), Erenpreiss et al. (2001) and Chohan et al. (2006) were higher than present observations.

4.3.3.2 DNA damage (single gel electrophoresis- Comet assay)

From table 1 it is evident that no DNA damage was detected in Osmanabadi and Sirohi bucks neat semen using Comet assay.

The literature pertaining to bucks semen particularly for DNA damage in neat semen detected by Comet assay was scanty; hence the comparison with other species was also made.
The observation of Li et al. (2007) was in close agreement with present observation who also reported 0 % DNA damage in fresh semen of Rhesus monkey. Similar observation of low DNA damage was observed by Ibrahim et al. (2008) in Boer male goats semen.

Ding et al. (2004) reported only 1.9 percent DNA damage in human spermatozoa but Aravindan et al. (1997), Hughes et al. (1999) Donnelly et al. (2001), Singh et al. (2003) and Isachenko et al. (2004) reported 10 to 20 percent DNA damage in fresh human semen. These observations are higher than the DNA damage recorded in present study.

Fraser et al. (2006) in bulls recorded 20% DNA damage and Nandre (2007) recorded 5.3% DNA damage in buffalo bulls spermatozoa in fresh semen. These observations were higher than present study on bucks.

4.4 Enzymes in seminal plasma of Osmanabadi and Sirohi bucks.

The different enzyme activity of neat semen of Osmanabadi and Sirohi bucks semen is given in table 1

4.4.1 Transaminase (GOT and GPT)

From table 1 it is evident that Glutamic- Oxaloacetic Transaminase (GOT) in seminal plasma of Osmanabadi and Sirohi buck was 82.55±6.95 and 73.61±4.37 IU/L respectively.

There was no significant difference (P>0.05) between two breeds of bucks with respect to GOT in neat semen.

The observations recorded by Tuli and Holtz (1994) in Boer goats are in agreement with present findings. They observed 82.5±3.0 units/L GPT release in Boer goat semen which increased after freezing.
In present study higher activity of GOT than GPT was recorded in undiluted semen of Osmanabadi and Sirohi bucks, similar trend was observed by Chauhan et al. (1994) for Jamnapri bucks.

In present study higher activity of GOT than GPT was recorded in fresh semen, similar type of trend was observed by following authors in different species. Naforinita et al. (1977) in cow bulls, Chauhan and Srivastav (1973), Varshney et al. (1978), Dabas et al. (1982) in Murrah bulls, Yaqub et al.(1983) in Nill-Ravi buffalo bulls, Dhami (1986) Dhami and Kodagali (1988) in surati bulls and Kaker and Arora (1976) in crossbred cow bulls.

From table 5 it is evident that of Glutamic- Pyruvate Transaminase (GPT) in seminal plasma of Osmanabadi and Sirohi buck was 16.94 ±0.84 and 16.06±0.81 IU/L respectively.

There was no significant difference (P>0.05) between two breeds of bucks with respect to GPT in neat semen.

In present study lower level of GPT than GOT recorded was in both the breeds. Similar type of trend was observed by Chauhan et al. (1994) for Jamnapri bucks.

The following authors also observed lower activity of GPT than GOT in fresh semen of different species which was in agreement with present observations. Zahariew et al. (1974), in Bulgarian Brown bulls, Kaker and Arora (1976) in young Holstein Friesian X Hariana and Brown Swiss X Hariana cross – bred bulls, Yaqub et al. (1983) in Nill-Ravi buffalo bull, Naforinita et al..(1977) in bulls and Varshney et al. (1978) in Murrah buffalo bulls, Singal et al.(1976) in Hariyana with, Holstein Friesian or Brown Swiss bulls. Dabas et al. (1982) in Red Dane and Murrah bulls and Dhami (1986) in Surati bulls

Murdoch and White (1968) reported that if a particular enzyme is present in whole semen, it occurs in both spermatozoa and seminal plasma. They also observed GOT activity as 293±50 595±113. 2400±340, 90±159 and 16800±30300 unit/ml in ram, bull, dog, rabbit and human whole semen respectively. This indicates that there is variation in semen of different species for GOT activity.
4.4.2 Alkaline phosphatase (AKP) and Acid phosphatase (ACP)

From table 1 it is evident that average of AKP in seminal plasma of Osmanabadi and Sirohi buck was 970.9±5.39 and 974.1±4.24 IU/L respectively.

There was no significant difference (P>0.05) between two breeds of bucks with respect to AKP in neat semen.

From table 1 it is evident that average of ACP in seminal plasma of Osmanabadi and Sirohi buck was 48.95 and 47.38 IU/L respectively.

There was no significant difference (P>0.05) between two breeds of bucks with respect to ACP in neat semen.

Chauhan et al. (1994) in Jamanpardi buck as well as Murdoch and White (1968) in ram, bull and dog recorded higher levels of AKP than ACP in fresh semen this type of trend was observed in present study. The exact values were not compared due to difference in methods of estimation and unit of expression.

Following authors had also reported the higher levels of AKP than ACP in semen of cow bull or buffalo bull which was in agreement with present findings. Eapen et al. (1964), Misra et al. (1969) and Kumar et al. (1984) in Murrah buffalo bulls; Dhami (1986), Dhami and Kodagali (1988), Dhami et al. (1990) in Surti buffalo bulls. Ibrahim et al. (1985) in buffalo bulls. Kaker and Arora (1973) in HF x Hariana and BS x Hariana bulls, Naforanita et al. (1977) in bull semen, Following authors had reported the higher levels of ACP than AKP in semen of cow bull or buffalo bull which is not in agreement with present findings.


The above variation may be attributed to the species of animals, breed as Murdoch and White (1968) also reported great variation in activity of AKP and ACP in different species of animals.
4.4.3 Lactate Dehydrogenase (LDH)

From table 1 it is evident that LDH in seminal plasma of Osmanabadi and Sirohi buck was 293.4±9.28 and 281.6±6.43 IU/L respectively.

There was no significance difference (P>0.05) between two breeds of bucks with respect to LDH in neat semen.

Dhami (1986), Dhami and Kodagali (1988) observed 364 to 395 IU/L LDH in Surti buffalo bulls which was slightly higher than present observations.

The following authors had recorded LDH activity in neat semen of different species but due to difference of expression in units the comparison could not be made.


Zamiri and Heidari (2006) also recorded LDH in Rayini male goats of Kerman province in Iran but the actual values were not comparable because of difference in units.

As Murdoch and White (1968) stated that they observed great variation in activity of LDH in different species of animals, which may be the case with present observations.

When activity of different enzymes (GOT, GPT, AKP, ACP and LDH) in the seminal plasma of Osmanabadi and Sirohi bucks studied, references were not available. Therefore, comparison was not possible. Sometimes references about other species were available but the ‘units’ of expression were not similar. Therefore such references could not be compared.
4.5 Evaluation of semen quality of Osmanabadi and Sirohi bucks at different freezing stages in different dilutors.

4.5.1 Sperm Motility

The sperm motility percentage at different freezing stages in different dilutors is given in table no 3.

Table 3 Sperm motility percentage at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks (Mean±S.E, n=18)

<table>
<thead>
<tr>
<th>Dilutors</th>
<th>Sperm motility (%)</th>
<th>C.V</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-freezing</td>
<td></td>
<td>5.265</td>
<td>13.889*</td>
</tr>
<tr>
<td>TYG(O)</td>
<td>82.22±1.73*ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD(O)</td>
<td>83.33±2.11*a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE(O)</td>
<td>77.78±2.68*cd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG(S)</td>
<td>71.66±1.43*er</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (S)</td>
<td>74.45±1.65*cdde</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE(S)</td>
<td>66.65±1.72*h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-freezing 24 hr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG (O)</td>
<td>77.22±2.34*cd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (O)</td>
<td>78.38±2.04*bc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE (O)</td>
<td>72.22±1.85*e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG (S)</td>
<td>67.77±1.11*fgh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (S)</td>
<td>72.76±1.02*e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE (S)</td>
<td>64.43±2.04*hi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-freezing 72 hr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG (O)</td>
<td>73.89±2.00*de</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (O)</td>
<td>75.00±1.70*ede</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE (O)</td>
<td>71.11±1.11*efg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG (S)</td>
<td>67.23±1.02*gh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (S)</td>
<td>72.20±0.70*e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE (S)</td>
<td>61.66±1.13*i</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

O=Osmanabadi S= Sirohi, *significant at 5% level, CD (0.05) = 4.391. Means with common superscript do not differ significantly.
**4.5.1.1 Sperm motility percentage at pre-freezing (5°C) stage**

From table 3 it is evident that the average sperm motility percentage of semen at pre-freezing (5°C) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 82.22±1.73, 83.33±2.11 and 77.78±2.68 and for Sirohi bucks it was 71.66±1.43, 74.45±1.65 and 66.65±1.72 respectively.

There was a significant difference (P<0.05) between two breeds of bucks with respect to sperm motility percentage at pre-freezing stage. The sperm motility was significantly higher in Osmanabadi than Sirohi bucks.

There was a significant difference (P<0.01) across different dilutors with respect to sperm motility percentage at pre-freezing stage.

The results further indicated that there was a significant difference between TYG – TYE and TYD- TYE dilutors with respect to sperm motility percentage in both the breeds. Sperm motility was significantly higher in TYD and TYG dilutors than TYE dilutor for both breeds. No significant difference in sperm motility percentage was noted in TYG and TYD dilutors in both the breeds.

Sperm motility recorded at pre-freezing stage (5°C) between 60 to 80 percent by following authors was in close agreement with present findings.

Deka and Rao (1986) in Indigenous goat breeds from Andrapradesh (65.05%), Tuli and Holtz (1992) in Boer goat (65.25 ± 1.74 %), Tuli and Holtz (1994) in Boer goat (69.0± 2.4%), Khalifa and Saidy (2006) in Damascus bucks (81.67±1.18) and El-Kon et al. (2010) in Damascus bucks (65.75±0.59%).

However, sperm motility recorded by Dunner (1993) in Spanish bucks was much lower than present observations (39 to 54 %) which could be due to difference in breed, climate and dilutor of semen.
4.5.1.2 Sperm motility percentage at post-freezing (24 hours) stage

From table 3 it is evident that the average sperm motility percentage of semen at post-freezing (24 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks is 77.22±2.34, 78.38±2.04, 72.22±1.85 and Sirohi bucks is 67.77±1.11, 72.76±1.02 and 64.43±2.04 respectively.

There was a significant difference (P<0.05) between two breeds of bucks with respect to sperm motility percentage. Sperm motility was significantly higher in Osmanabadi than Sirohi bucks at 24 hours post-freezing stage.

There was a significant difference across different dilutors with respect to sperm motility percentage at post-freezing (24 hours) stage.

The results further indicated that in Osmanabadi bucks semen there was a significant difference between TYG and TYE as well as TYD and TYE dilutors with respect to sperm motility percentage at post-freezing (24 hours) stage. Sperm motility was significantly higher in TYD and TYG than TYE dilutor. There was no significant difference in sperm motility percentage in TYG-TYE in Osmanabadi bucks.

In Sirohi bucks semen the sperm motility was higher in TYD as compared to TYG and TYE dilutor in present study.

In both the breeds TYD dilutor shows better sperm motility, this indicates that DMSO was better cryo-protectant for preserving buck semen motility post-freezing. Similarly, Kashiwazaki et al. (2006) for Japanese White rabbit spermatozoa observed that in frozen thawed spermatozoa the rate of forward progressive motile spermatozoa in DMSO (37.8 ± 3.0%) was significantly (P<0.05) higher than in glycerol (17.0 ± 3.3%), which indicated that DMSO was comparatively better cryoprotectant than glycerol. This trend was similar to trend observed in present study.

The post thaw sperm motility at 24 hours after freezing was reported in the range of 60 to 80 percent by following scientist which was in close agreement with present findings.

The post thaw sperm motility observed less than 50 percent by following authors which was lower than present observations.


The post thaw sperm motility depends on removal or non-removal of seminal plasma, Tuli and Holtz (1994) obtained a considerably higher percentage of motile spermatozoa and observed that there was no favorable effect of the removal of semen plasma which was in agreement with our observations, where we did not remove the seminal plasma prior to dilution of semen.

4.5.1.3 Sperm motility percentage at post-freezing (72 hours) stage

From table 3 it is evident that the average sperm motility percentage of semen at post-freezing (72 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 73.89±2.00, 75.00±1.70 and 71.11±1.11 and for Sirohi bucks it was 67.23±1.02, 72.20±0.70 and 61.66±1.13 respectively.

There was a significant difference (P<0.05) between the two breeds of bucks with respect to post-freezing (72 hours) sperm motility percentage in TYG and TYE dilutor. The sperm motility was significantly higher in Osmanabadi than Sirohi bucks in TYG and TYE dilutors. The sperm motility in TYD dilutor was not significantly different in both breeds indicating good sperm viability in TYD dilutor.

There was a significant difference (P<0.05) across different dilutors with respect to post-freezing (72 hours) sperm motility percentage.

The results further indicated that in Osmanabadi bucks no significant difference in sperm motility was observed across three dilutors.

In Sirohi bucks there was a significant difference between TYG-TYD, TYD-TYE and TYG-TYE dilutors with respect to post-freezing (72 hours) sperm motility percentage. The sperm motility reduced significantly in TYE than TYG and TYD dilutor. This indicates that ethylene glycol was not providing optimum cryo-protecting environment to bucks spermatozoa at post-freezing stage. The sperm motility was also significantly low in TYG as compared to TYD dilutor.
Deka and Rao (1986) in indigenous bucks from Andrapradesh recorded 64.0%, 66.92% and 63.65 % post thaw sperm motility when semen was frozen with 4, 6.4 and 9 % glycerol. These findings were within agreement with present observations for TYG dilutor. Anel et al. (2003) observed 75.8 percent post thaw sperm motility in ram semen which in agreement with present study for both the breed. Kashiwazaki et al. (2006) observed that frozen thawed Japanese White rabbit spermatozoa the rate of forward progressive motile spermatozoa in DMSO was significantly (P<0.05) higher than in glycerol. Similar observations were recorded in present study also.


### 4.5.1.4 Sperm motility percentage in TYG dilutor

From table 3 it is evident that sperm motility percentage of semen in TYG dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stage for Osmanabadi bucks was 82.22±2.04, 77.22±2.34 and 73.89±2.00 and Sirohi bucks it was 71.66±1.42, 67.77±1.11 and 67.23±1.02 respectively.

There was a significant difference (P<0.05) between the two breeds of the bucks with respect to sperm motility percentage in TYG dilutor at pre- freezing and post-freezing 24 and 72 hours stages. Sperm motility was significantly higher in Osmanabadi than Sirohi bucks at all the three stages of freezing in TYG dilutor.

There was a significant difference (P<0.05) across different time intervals and temperatures with respect to sperm motility percentage in TYG diluter.

In Osmanabadi bucks there was a significant difference between pre-freezing and 24 hours post-freezing, pre-freezing and 72 hours post-freezing sperm motility percent. Sperm motility was significantly reduced from pre-freezing to post freezing (24 hours) stage in Osmanabadi bucks.
In Sirohi bucks no significant difference in sperm motility was noted between pre-freezing and post freezing (24 hours) stage, but sperm motility was significantly reduced at 72 hours post freezing as compared to pre freezing stage.

As there was no significant difference noted in sperm motility percentage between post-freezing 24 and 72 hours for both the breeds we can conclude that sperm motility was stable within normal limits in TYG dilutor after freezing of semen.

Similar findings were obtained by Deka and Rao (1986) in indigenous bucks from Andhra Pradesh who recorded 64.0%, 66.92% and 63.65 % post thaw sperm motility when semen was frozen with 4, 6.4 and 9 % glycerol in dilutor. But Tuli and Holtz (1994) recorded only 38.5 ± 2.7 % progressive sperm motility after thawing in Boer bucks semen in Tris egg yolk glycerol dilutor with one step freezing procedure. This variation may be due to percentage of egg yolk or glycerol used. The findings recorded by Kundu et al. (2000) supports that higher percentage of glycerol was not desirable in buck semen preservation. They observed that addition of glycerol (0.22 to 0.87 M) caused a dose-dependent increase of post thaw sperm motility. The highest forward and total motility (32 and 35%, respectively) was at 0.87 M. Further increase of the glycerol caused a marked decrease in motility. Kundu et al. (2002) also specified that Dextran served as a significant cryoprotectant in the presence of glycerol (0.87 mol l (-1)) and dimethyl sulphoxide (0.76 mol l (-1)). In the presence of the three cryoprotectants (Dextran, Glycerol and DMSO), recovery of sperm motility was as high as 58% (forward motility) and 60% (total motility).

Eiman and Takato (2004) in a trial using Tris-egg-yolk-glycerol and trehalose-egg yolk- glycerol observed 73± 0.9 % and 57.0±1.8% post thaw sperm motility respectively in bucks. These observations also support the present findings.
4.5.1.5 Sperm motility percentage in TYD dilutor

From table 3 it is evident that sperm motility percentage of semen in TYD dilutors at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stage for Osmanabadi bucks was 83.33±2.11, 78.38±2.04 and 75.00±1.85 and for Sirohi bucks it was 74.45±1.64, 72.76±1.02, and 72.20±2.04 respectively.

There was a significant difference (P<0.05) between the two breeds of bucks with respect to sperm motility percentage at pre-freezing and post freezing (24 and 72 hours) stages. Significantly higher sperm motility observed was in Osmanabadi than Sirohi bucks at pre-freezing than post freezing (24 hours) stage. No significant difference in sperm motility observed at post freezing (72 hours) stage in both the breeds.

There was a significant difference (P<0.05) across different time interval and temperature with respect to post thaw sperm motility in TYD dilutor.

The results further indicated that In Osmanabadi bucks there was a significant difference between pre-freezing and post-freezing (24 hours) as well as pre-freezing and post-freezing (72 hours) stages with respect to sperm motility percentage in TYD dilutor. Sperm motility was significantly reduced at post freezing (24 hours) and post freezing (72 hours) stage than pre-freezing stage. Sperm motility was not significantly reduced from post freezing (24 hours) to post freezing (72 hours) stage.

In Sirohi bucks no significant difference noted in sperm motility percentage between different time intervals and temperature in TYD dilutor.

There was no significant difference noted in sperm motility percentage between post-freezing (24 hours) and post freezing (72 hours) stage in both the breeds. Thus we can conclude that sperm motility was stable in TYD dilutor after freezing.

Similar to present study Kundu et al. (2000) has also used DMSO as cryoprotectant to preserve goat cauda epididymal sperm and observed high sperm motility in dimethyl sulfoxide. Kundu et al. (2002) observed that “Dextran” served as a significant cryoprotectant in the presence of dimethyl sulphoxide. In the presence of
the three cryoprotectants (Dextran, Glycerol and DMSO) sperm motility was 58% (forward motility) and 60% (total motility).

Kashiwazaki et al. (2006) studied effect of glycerol and dimethyl sulfoxide (DMSO) as cryoprotectants for Japanese White rabbit spermatozoa. In frozen thawed spermatozoa the rate of forward progressive motile spermatozoa in DMSO (37.8 ± 3.0%) was significantly (P<0.05) higher than in glycerol (17.0 ± 3.3%) which was in agreement with present observations for bucks.

4.5.1.6 Sperm motility percentage in TYE dilutor

From table 3 it is evident that the average sperm motility percentage in TYE dilutors at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stage for Osmanabadi bucks was 77.78±2.67, 72.22±1.85 and 71.11±1.11 and for Sirohi bucks it was 66.65±1.72, 64.43±2.04, and 61.66±1.13 respectively.

There was a significant difference (P<0.05) between two breeds of bucks with respect to sperm motility percentage at pre freezing and post freezing(24 and 72 hours) stages in TYE dilutor. Significantly higher sperm motility percentage observed was in Osmanabadi than Sirohi bucks.

There was a significant difference (P<0.05) across different dilutors with respect to sperm motility percentage in TYE dilutor.

The results further indicated that in Osmanabadi bucks semen there was a significant difference between pre-freezing and post-freezing (24 and 72 hours) sperm motility percentage in TYE dilutor. Sperm motility significantly reduced at 24 and 72 hours post-freezing than pre-freezing stage. No significant reduction in sperm motility noted between post-freezing stages. It indicates that sperm motility was stable post-freezing for Osmanabadi breed semen in TYE dilutor.

In Sirohi bucks semen there was no significant difference between pre-freezing and post-freezing (24 hours) as well as 24 hours post freezing and 72 hours post freezing sperm motility percentage in TYE dilutor. There was significant reduction in sperm motility was observed from pre freezing to post freezing (72 hours) stage.
Kundu et al. (2000) observed ethylene glycol had markedly lower cryoprotection potential than glycerol, which is in agreement with present findings as the sperm motility was lower in TYE dilutor than TYD and TYG dilutors.
### 4.5.2 Live sperm percentage

The live sperm percentage at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks semen is given in table 8.

Table 4 Live sperm percentage at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks (Mean±S.E, n=18)

<table>
<thead>
<tr>
<th>Dilutor</th>
<th>Live sperm percentage (%)</th>
<th>C.V</th>
<th>F-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-freezing Average</td>
<td>2.812</td>
<td>7.037*</td>
<td></td>
</tr>
<tr>
<td>TYG(O)</td>
<td>81.99±0.51&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.037*</td>
<td></td>
</tr>
<tr>
<td>TYD(O)</td>
<td>82.94±0.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE(O)</td>
<td>78.55±1.07&lt;sup&gt;cde&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG(S)</td>
<td>80.44±1.27&lt;sup&gt;abc&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (S)</td>
<td>81.66±1.25&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE(S)</td>
<td>79.88±1.24&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-freezing 24 hr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG (O)</td>
<td>78.55±0.70&lt;sup&gt;cde&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (O)</td>
<td>78.77±1.52&lt;sup&gt;cde&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE (O)</td>
<td>75.66±0.95&lt;sup&gt;efg&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG (S)</td>
<td>78.77±1.38&lt;sup&gt;cde&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (S)</td>
<td>79.66±1.36&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE (S)</td>
<td>76.55±1.68&lt;sup&gt;efg&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-freezing 72 hr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG (O)</td>
<td>77.83±0.84&lt;sup&gt;def&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (O)</td>
<td>77.50±0.75&lt;sup&gt;def&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE (O)</td>
<td>74.05±0.52&lt;sup&gt;g&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG (S)</td>
<td>77.61±1.29&lt;sup&gt;def&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (S)</td>
<td>78.22±1.41&lt;sup&gt;cde&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE (S)</td>
<td>74.72±1.66&lt;sup&gt;g&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

O=Osmanabadi S= Sirohi, *Significant at 5% level, CD (0.05) = 2.534. Means with common superscript do not differ significantly.
4.5.2.1 Live sperm percentage at pre-freezing (5°C) stage

From table 4 it is evident that the average live sperm percentage of semen at pre-freezing (5°C) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 81.99±0.51, 82.94±0.76 and 78.55±1.07 and for Sirohi bucks it was 80.44±1.27, 81.66±1.25 and 79.88±1.24 respectively.

There was no significant difference (P>0.05) between the two breeds of the bucks with respect to live sperm percentage.

The results further indicated that in Osmanabadi bucks semen there was a significant difference between TYG –TYE and TYD-TYE dilutor with respect to live sperm percentage. Live sperm percentage was significantly higher in TYG and TYD than TYE dilutors. No significant difference in live sperm percentage was noted in TYG-TYD dilutors.

In Sirohi bucks semen no significant difference in live sperm percentage was noted in TYG-TYD, TYG-TYE as well as TYD-TYE dilutors.

The observations recorded in semen for live sperm percentage at pre-freezing stage (5°C) by Tuli and Holtz (1994) in Boer bucks (74.4±3.0), Rizal et al. (2002) in Garut ram (77.00%), Selarnet et al. (2006) in Garut ram (75.00 ± 3.52), were in agreement with present observations. But similar types of observation recorded by Tuli and Holtz (1992) in Boer goats (54.85±3.71 to 60.20±3.35) Fernandez-Santos et al. (2006) in Red Deer epididymal spermatozoa (50 to 70%) were lower than present observations; this could be attributed to cryoprotectant, dilutor used, breed, age etc

4.5.2.2 Live sperm percentage at post-freezing (24 hours) stage

From table 4 it is evident that the average live sperm percentage in semen at post-freezing (24 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 78.55±0.70, 78.77±1.52 and 75.66±0.95 and for Sirohi bucks it was 78.77±1.38, 79.66±1.36 and 76.56±1.68 respectively.
There was no significant difference (P>0.05) observed between the two breeds as well as across different dilutors with respect to live sperm percentage. Live sperm percentage was non-significantly higher in Sirohi than Osmanabadi bucks at post-freezing (24 hours) stage.

Results in agreement with present observations were obtained by Anel et al. (2003) in ram semen (68.7%).

Lower post thaw live sperm percent than present observation were observed by Tuli and Holtz (1992) in Boer bucks (26.40 ± 2.78 to 34.25 ± 2.50), Tuli and Holtz (1994) in Boer bucks (42.3±2.9%) Selarnet et al. (2006) in Garut ram (55.67±3.08) and Umut and Daskin (2010) in Angora bucks (61%).

This difference in post thaw viability may be attributed to the different cooling rates (Ritar and Ball 1993), choice of cryopreservation methods used, handling of semen, labor and inventory management.

### 4.5.2.3 Live sperm percentage at post-freezing (72 hours) stage

From table 4 it is evident that the average live sperm percentage of semen at post-freezing (72 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 77.83 ± 0.84, 77.50 ± 0.75 and 74.05 ± 0.52 and for Sirohi bucks it was 77.61 ± 1.29, 78.22 ± 1.41 and 74.72 ± 1.66 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to post-freezing (72 hours) live sperm percentage.

There was a significant difference (P<0.05) across different dilutors with respect to live sperm percentage.

The results further indicated that in Osmanabadi and Sirohi bucks semen there was a significant difference between dilutors TYG and TYE as well as TYD and TYE dilutors at post-freezing (72 hrs) stage with respect to live sperm percentage. Significantly higher live sperm percentage noted in TYG and TYD than TYE dilutors. There was no significant difference in TYG and TYD dilutors with respect to live sperm
percentage. This indicates that glycerol and DMSO were better than ethylene glycol for preserving sperm viability in Osmanabadi and Sirohi bucks semen at post-freezing (72 hours) stage.

Tuli and Holtz (1992) in Boer bucks recorded live sperm percentage as 26.40±2.78 to 34.25±2.50 which was lower than present observations. This may be attributed to cryoprotectant used. But still they opined that semen samples when extended with Tris yolk glycerol showed significantly (P<0.01) higher live spermatozoa when extended with the other zwitterions buffer-based extenders, this type of observations are in agreement with trend recorded in present study.

**4.5.2.4 Live sperm percentage TYG dilutor**

From table 4 it is evident that the average live sperm percentage of semen in TYG dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stage for Osmanabadi bucks was 81.99±0.51, 78.55±0.69 and 77.83±0.84 and for Sirohi bucks it was 80.44±1.26 79.66±1.38, 78.22±1.29 respectively.

There was no significant difference (P>0.05) with respect to live sperm percentage at pre and post freezing stages in both the breeds.

There was a significant difference (P<0.05) across different time intervals and temperatures with respect to live sperm percentage in TYG dilutor.

The results further also indicated that in Osmanabadi bucks there was a significant difference between pre-freezing and 24 hours post- freezing as well as pre-freezing and 72 hours post-freezing stage in TYG dilutor with respect to live sperm percentage. Live sperm percent reduced significantly from pre freezing to post freezing (24 and 72 hours) stage in Osmanabadi bucks semen.

In Sirohi bucks semen there was no significant reduction in live sperm percent from pre freezing to post freezing (24 and 72 hours) stage.

As there was no significant difference noted in live sperm percentage between post-freezing 24 and 72 hours in both the breeds we can conclude that sperm viability was stable in TYG dilutor after freezing.
Tuli and Holtz (1992) in Boer bucks opined that semen samples when extended with Tris yolk glycerol showed significantly ($P<0.01$) higher live spermatozoa than extended with the other zwitterions buffer-based extenders. Similar types of results are observed in present study that higher live sperm percentage was observed in TYG dilutor than TYE dilutor. The observations recorded by Fernandez-Santos et al. (2006) in Red Deer epididymal spermatozoa at pre freeze stage in 6% glycerol dilutor (60-70% live sperm) was also near to agreement with present observations. From this we can opined that glycerol and DMSO were better cryoprotectant than ethylene glycol for Osmanabadi and Sirohi buck semen.

### 4.5.2.5 Live sperm percentage TYD dilutor

From table 4 it is evident that the average live sperm percentage of semen in TYD dilutors at pre-freezing ($5^\circ$C), post-freezing (24 hours) and post-freezing (72 hours) stage for Osmanabadi bucks was 82.94±0.76, 78.77±1.52 and 77.50±0.75 and for Sirohi bucks it was 81.66±1.25, 79.66±1.36 and 78.22±1.41 respectively.

There was no significant difference ($P>0.05$) between the two breeds of bucks with respect to live sperm percentage in TYD dilutor at different time interval and temperature.

There was a significant difference ($P<0.05$) across different time interval and temperature with respect to live sperm percentage in TYD dilutor.

The results further indicated that in Osmanabadi bucks semen there was a significant difference between pre-freezing and post-freezing (24 hours) as well as pre-freezing and post-freezing (72 hours) stage with respect to live sperm percentage in TYD dilutor. Live sperm percentage was significantly reduced at post freezing 24 and 72 hours than pre-freezing stage.

In Sirohi bucks no significant difference noted in live sperm percentage in TYD dilutor at different time interval and temperature except pre freezing to post freezing (72 hours) stage. Significant reduction in live sperm percent was recorded from pre freezing to post freezing (72 hours) stage.

As there was no significant difference noted between 24 and 72 hours post-freezing stage in both the breeds with respect to live sperm percentage that indicates
that sperm viability was not affected after 24 hours of freezing. It means that sperm viability was better preserved in TYD dilutor for both the breeds.

On scanning the available literature, the references regarding live sperm percentage of semen of Osmanabadi and Sirohi bucks in TYD dilutor at different time interval and temperatures were not available. Therefore no comparison could be made.

4.5.2.6 Live sperm percentage TYE dilutor

From table 4 it is evident that the average live sperm percentage in TYE dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stage for Osmanabadi bucks was 78.55 ± 1.07, 75.66±0.95 and 74.05±0.52 and for Sirohi bucks it was 79.88±1.24, 76.55±1.68 and 74.72±1.65 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to live sperm percentage in TYE dilutor.

There was a significant difference (P<0.05) across different time interval and temperature with respect to live sperm percentage in TYE dilutor.

The results further indicated that in Osmanabadi bucks there was a significant difference between pre-freezing and post freezing (72 hours) stage with respect to live sperm percentage in TYE dilutor. Live sperm percentage significantly reduced at post freezing (72 hours) stage than pre freezing stage. No significant difference was noted between pre freezing and post freezing (24 hours) as well as post freezing (24 hours) and post freezing (72 hours) stage.

In Sirohi bucks there was significant difference between pre-freezing and post-freezing (24 hours) as well as pre-freezing and 72 hours post-freezing live sperm percent in TYE dilutor. Live sperm percentage significantly reduced post-freezing (24 and 72 hours) than pre-freezing stage.

In both the breeds, no significant difference noted between 24 and 72 hours post-freezing stages. This indicates stable sperm viability after freezing.

On scanning the available literature, the references regarding live sperm percentage in TYE dilutor post freezing in semen of Osmanabadi and Sirohi bucks in TYE dilutor at different time interval and temperatures were not available. Therefore no comparison could be made.
The abnormal sperm percentage at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks semen is given in table 9.

<table>
<thead>
<tr>
<th>Dilutor</th>
<th>Abnormal sperm (%)</th>
<th>C.V</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-freezing</td>
<td>(Mean±S.E, n=18)</td>
<td>9.960</td>
<td>9.212*</td>
</tr>
<tr>
<td>TYG(O)</td>
<td>16.60±0.45efg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD(O)</td>
<td>16.11±0.49fgh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE(O)</td>
<td>17.88±0.73def</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG(S)</td>
<td>14.33±0.73h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD(S)</td>
<td>15.72±0.95gh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE(S)</td>
<td>16.61±1.03efg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-freezing 24 hr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG(O)</td>
<td>18.61±0.52cde</td>
<td>c</td>
<td></td>
</tr>
<tr>
<td>TYD(O)</td>
<td>17.50±0.65efg</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td>TYE(O)</td>
<td>19.77±0.31ed</td>
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<td></td>
</tr>
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<td>TYG(S)</td>
<td>17.55±1.05efg</td>
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<td></td>
</tr>
<tr>
<td>TYD(S)</td>
<td>18.05±1.14def</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE(S)</td>
<td>19.99±1.20ed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-freezing 72 hr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>72hrs TYG(O)</td>
<td>20.22±0.52bc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72hrs TYD(O)</td>
<td>17.88±0.65def</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72hrs TYE(O)</td>
<td>22.22±0.31ab</td>
<td></td>
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<tr>
<td>72hrs TYG(S)</td>
<td>19.72±1.05ed</td>
<td></td>
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<tr>
<td>72hrs TYD(S)</td>
<td>19.72±1.14ed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72hrs TYE(S)</td>
<td>23.38±61.20a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

O=Osmanabadi S=Sirohi, * Significant at 5% level, C.D. (0.05) = 2.110 Means with common superscript do not differ significantly.
4.5.3.1 Abnormal sperm percentage at pre-freezing (5ºC) stage

From table 5 it is evident that average abnormal sperm percentage of semen at pre-freezing (5ºC) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 16.60±0.45, 16.11±0.49 and 17.88±0.73 and for Sirohi bucks it was 14.33±0.73, 15.72±0.95 and 16.61±1.03 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to abnormal sperm percentage except TYG dilutor. Significantly higher abnormal sperm percentage noted in Osmanabadi than Sirohi bucks at pre-freezing stage in TYG dilutor.

There was no significant difference (P<0.01) across different dilutors with respect to abnormal sperm percentage except TYG-TYE in Sirohi bucks. Significantly higher percentage of abnormal spermatozoa was noted in TYE than TYG dilutor in Sirohi bucks.

As there was no significant difference noted in sperm abnormality in different dilutors we can conclude that conditions in dilutors were optimum to preserve sperm morphology at pre-freezing stage.

The observations recorded about sperm abnormality at pre-freezing stage (5ºC) by Rohilla et al. (2005) in Murrah buffalo bulls semen (6.50±0.04% sperm abnormality in 5% ethylene glycol dilutor and 7.00±0.05 % sperm abnormality in 6.8% glycerol dilutor), Nandre (2007) in Surati bulls (Tris glycerol dilutor-3.91 ± 0.40% sperm abnormality) were lower than present observations.

As per the findings of Batista et al. (2010) in Majorera Bucks the sperm abnormality was not changed in Tris based dilutor when preserved at 4ºC, which is in agreement with present observations. In present observations also no significant change was noted in sperm morphology in all three dilutors at pre-freezing stage (5ºC).

However, compared with observation of Ari et al. (2011) in Tushin ram semen (49.50 to 65.7 sperm abnormality) the present findings show much encouraging results. This could be attributed to cryoprotectant efficacy in preserving the sperm morphology.
4.5.3.2 Abnormal sperm percentage at post-freezing (24 hours) stage

From table 5 it is evident that the average abnormal sperm percentage of semen at post-freezing (24 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 18.61±0.52, 17.50±0.65 and 19.77±0.31 and for Sirohi bucks it was 17.55±1.05, 18.05±1.14 and 19.99±1.20 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to abnormal sperm percent.

There was significant (P<0.05) difference across different dilutors with respect to abnormal sperm percentage at post-freezing (24 hours) stage.

The results further indicated that in Osmanabadi bucks there was significant difference between TYD and TYE dilutors with respects to abnormal sperm percent. Significantly higher abnormal sperm percent was noted in TYE than TYD dilutor. There was no significant difference in TYG and TYD as well as TYG and TYE dilutors with respect to abnormal sperm percent at post freezing (24 hours) stage.

In Sirohi bucks significant difference was observed in sperm abnormality between TYD and TYE as well as TYG and TYE dilutors. Significantly higher sperm abnormalities were recorded in TYE dilutor as compared to TYG and TYD dilutors.

As per the present analogy of not washing buck semen before freezing Azerêdo et al. (2001) opined that in bucks presence or absence of seminal plasma, for both fresh and frozen sample does not affect the sperm morphology significantly.

Lower sperm abnormalities than present observations were reported by Rohilla et al. (2005) in Murrah a buffalo bulls (6.50 ± 0.04 and 9.30 ± 0.03%) in 5% ethylene glycol than in 6.8% glycerol (7.00 ± 0.05 and 10.30 ± 0.07%), Nandre (2007) in surati bulls (4.91 ± 0.49%). Very high sperm abnormalities were observed by Ari et al. (2011) in Tushin ram semen (49.50 to 65.7 percent) than present observations. These types of variations in sperm morphology are observed due to different types of extenders used, handling of semen, and season of semen collection. Since the finding of present study is within the normal range (Less than 20 % sperm abnormalities) it can be concluded that all three dilutors were effective in preserving post thaw sperm morphology.
4.5.3.3 Abnormal sperm percentage at post-freezing (72 hours) stage

From table 5 it is evident that the average abnormal sperm percentage of semen at post-freezing (72 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was $20.22 \pm 0.52$, $17.88 \pm 0.65$ and $22.22 \pm 0.31$ and for Sirohi bucks it was $19.72 \pm 1.05$, $19.72 \pm 1.14$ and $23.38 \pm 1.20$ respectively.

There was no significant difference (P>0.05) between the two breeds with respect to abnormal sperm percentage at post-freezing (72 hours) stage. The results further indicated that there was a significant difference (P<0.05) across different dilutors with respect to abnormal sperm percentage.

In Osmanabadi bucks there was significant difference between TYD and TYE as well as TYG and TYD dilutor with respect to abnormal sperm percentage at post-freezing (72 hours) stage. Abnormal sperm percent were significantly higher in TYE than TYD as well as in TYG than TYD dilutor, which indicates that DMSO was better cryoprotectant than ethylene glycol and glycerol for Osmanabadi bucks semen preservation. There was no significant difference in TYG and TYE dilutor with respect to abnormal sperm percent.

In Sirohi bucks there was significant difference between TYG and TYE as well as TYD and TYE dilutors with respect to abnormal sperm percent at post-freezing (72 hours) stage. Significantly higher abnormal sperm percent recorded was in TYE than TYG and TYD dilutor which indicates DMSO and glycerol as better cryoprotectant than ethylene glycol for Sirohi bucks semen preservation. All the abnormalities observed in both the breeds were within the normal prescribed limit.

Bakshi (1991) recorded 9.18 percent abnormal sperm percentage in semen of Osmanabadi bucks in Tris extender at post-freezing (-196°C, 96 hours) stage, this was lower than present observations.

Bakshi (1991) also recorded average abnormal sperm percentage of Beetal, Saanen, Alpine crossbreds and Saanen crossbred semen preserved at -196°C in tris extender as 8.88, 9.63, 8.30 and 9.60 respectively. These findings were also lower than present observations.
4.5.3.4 Abnormal sperm percentage in TYG dilutor

From table 5 it is evident that the average abnormal sperm percentage of semen in TYG dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stage for Osmanabadi bucks was 16.60±0.45, 18.61±0.62 and 20.22±0.40 and for Sirohi bucks it was 14.33±0.86, 17.55±1.23 and 19.72±1.53 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks at post freezing stages but there was significant difference (P<0.05) at pre-freezing stage with respect to abnormal sperm percentage in TYG dilutor. Osmanabadi bucks semen showed significantly higher sperm abnormalities than Sirohi bucks at pre freezing stage in TYG dilutor.

There was a significant difference (P<0.05) across different time interval and temperature with respect to abnormal sperm percentage in TYG dilutor.

The results further indicated that in Osmanabadi bucks there was a significant difference between pre-freezing and post freezing (72 hours) stage with respect to abnormal sperm percent in TYG dilutor. Sperm abnormalities increased significantly at post freezing (72 hours) stage than pre freezing in Osmanabadi bucks. Since there was, no significant difference noted in abnormal sperm percentage between post-freezing 24 and 72 hours in Osmanabadi bucks, we could conclude that sperm abnormality was not increased due to freezing in TYG dilutor.

In Sirohi bucks, there was a significant difference between pre-freezing, 24 and 72 hours post-freezing as well as post freezing 24 and 72 hours stage in TYG dilutor with respect to abnormal sperm percentage. Sperm abnormalities increased significantly from pre freezing to post freezing (24 and 72 hours) as well as post freezing 24 to 72 hours. In Sirohi bucks although there was significant increase in sperm abnormalities from post freezing 24 to 72 hours stage with we can observe that the sperm abnormality were within prescribed normal limit at post freezing (72 hours) stage.

Present observations were higher than those recorded by Nandre (2007) who observed on preservation of semen of Surti buffalo bulls in Tris glycerol dilutor at pre-freeze and post-freeze stages, the abnormal sperm count was 3.91 ± 0.40% and 4.91 ± 0.49%. In winter the abnormal sperm count was 3.72 ± 0.52% and 3.94 ± 0.50% and
in summer it was $4.11 \pm 0.61\%$ and $5.88 \pm 0.79\%$. This type of variations was expected due to species difference.

### 4.5.3.5 Abnormal sperm percentage in TYD dilutor

From table 5 it is evident that the average abnormal sperm percentage of semen in TYD dilutors at pre-freezing ($5^\circ$C), post-freezing (24 hours) and post-freezing (72 hours) stage for Osmanabadi bucks was $16.11\pm0.49$, $17.50\pm0.76$ and $17.88\pm0.84$ and for Sirohi bucks it was $15.72\pm0.95$, $18.05\pm1.35$ and $19.72\pm1.27$ respectively.

There was no significant difference ($P>0.05$) between the two breeds of bucks with respect to abnormal sperm percentage at different time interval and temperatures in TYD dilutor.

There was a significant difference ($P<0.05$) across different time interval and temperatures with respect to abnormal sperm percentage in TYD dilutor.

The results further indicated that in Osmanabadi bucks semen there was a significant difference between pre-freezing and post freezing (72 hours) stage with respect to sperm abnormalities. Sperm abnormalities increased significantly at post freezing (72 hours) than pre freezing stage. But there was no significant increased in sperm abnormalities between 24 to 72 hours post freezing stage.

In Sirohi bucks semen there was a significant difference between pre-freezing and post freezing (24 and 72 hours) stage with respect to sperm abnormalities. Sperm abnormalities increased significantly at post freezing (24 and 72 hours) than pre freezing stage. But there was no significant increased in sperm abnormalities between 24 to 72 hours post freezing stage.

As there was no significant increase in sperm abnormalities from 24 to 72 hours post freezing stage in both the breeds we can opine that sperm morphology was not affected in TYD dilutor on frozen storage of semen.

On scanning the available literature, the references regarding abnormal sperm percentage of semen of Osmanabadi and Sirohi bucks in TYD dilutor at different time interval and temperatures were not available. Therefore no comparison could be made.
4.5.3.6 Abnormal sperm percentage in TYE dilutor

From table 5 it is evident that the average abnormal sperm percentage in TYE dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stage for Osmanabadi bucks was 17.88±0.73, 19.77±0.36 and 22.22±0.98 and for Sirohi bucks it was 16.61±1.03, 19.99±1.42 and 23.38±2.04 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to abnormal sperm percentage at different time interval and temperature in TYE dilutor. There was a significant difference (P<0.05) across different time interval and temperature with respect to abnormal sperm percentage in TYE dilutor.

The results further indicated that in Osmanabadi bucks there was a significant difference with respect to abnormal sperm percentage between pre-freezing and post-freezing (72 hours) stage in TYE dilutor. Sperm abnormality increased significantly at post freezing (72 hours) than pre freezing stage. No significant difference was noted between 24 and 72 hours post-freezing indicates that sperm abnormality was not increased on frozen storage.

In Sirohi bucks there was a significant difference with respect to abnormal sperm percentage between pre-freezing and post-freezing (24 hours) stage as well as pre-freezing and post-freezing (72 hours) stage in TYE dilutor. Sperm abnormality increased significantly at post freezing (24 and 72 hours) than pre freezing stages. Significant difference in abnormal sperm percentage was also noted between 24 and 72 hours post-freezing stages indicate that sperm abnormality was increasing as freezing time progressed. This indicates that TYE dilutor was not effective for protecting sperm morphology post freezing in Sirohi breed.

Rohilla et al. (2005) reported lower sperm abnormalities during pre- and post-freezing and thawing of semen were (6.50±0.04 and 9.30±0.03%) in dilutor with 5% ethylene glycol, these observations were lower than present observations. This could be attributed to difference in species of animals.
4.5.4 Plasma membrane integrity of spermatozoa (HOST)

The Plasma membrane integrity sperm percentage at different freezing stages in different dilutors of Osmanabadi and Sirohi bucks detected by Hypo-Osmotic Swelling Test (HOST) is given in table 10.

Table 6 Plasma membrane integrity percentage of spermatozoa at different freezing stages in different dilutors of Osmanabadi and Sirohi bucks (HOST)

<table>
<thead>
<tr>
<th>Dilutor</th>
<th>Sperm Plasma membrane Integrity (%)</th>
<th>C.V</th>
<th>F-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-freezing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG(O)</td>
<td>72.50±1.03&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.921</td>
<td>6.036*</td>
</tr>
<tr>
<td>TYD(O)</td>
<td>74.22±1.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE(O)</td>
<td>72.05±2.54&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG(S)</td>
<td>71.00±1.15&lt;sup&gt;bc&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD(S)</td>
<td>72.11±1.15&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE(S)</td>
<td>67.83±0.62&lt;sup&gt;de&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Post-freezing 24 hr.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG(O)</td>
<td>68.83±1.95&lt;sup&gt;cde&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD(O)</td>
<td>72.66±1.78&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE(O)</td>
<td>68.72±1.46&lt;sup&gt;cde&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG(S)</td>
<td>68.83±0.59&lt;sup&gt;cde&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD(S)</td>
<td>70.11±0.84&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td></td>
<td></td>
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<tr>
<td>TYE(S)</td>
<td>66.50±0.76&lt;sup&gt;ef&lt;/sup&gt;</td>
<td></td>
<td></td>
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<tr>
<td><strong>Post-freezing 72 hr.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG(O)</td>
<td>67.27±1.16&lt;sup&gt;de&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD(O)</td>
<td>70.16±1.42&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE(O)</td>
<td>67.83±1.23&lt;sup&gt;de&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG(S)</td>
<td>66.33±0.72&lt;sup&gt;ef&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD(S)</td>
<td>67.50±1.14&lt;sup&gt;de&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE(S)</td>
<td>63.66±1.10&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
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</tr>
</tbody>
</table>

O=Osmanabadi S= Sirohi, * Significant at 5% level, C.D (0.05) = 3.120 Means with common superscript do not differ significantly
4.5.4.1 Plasma membrane integrity of spermatozoa at pre-freezing (5°C) stage (HOST).

From table 6 it is evident that the average hypo-osmotic swelling sperm percentage of semen at pre-freezing (5°C) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 72.50±1.03, 74.22±1.03 and 72.05±2.54 and for Sirohi bucks it was 71.00±1.15, 72.11±1.15 and 67.83±0.62 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to HOS sperm percentage in TYG and TYD dilutors but significantly higher HOS sperm percent was noted in TYE dilutor for Osmanabadi than Sirohi bucks.

In Osmanabadi bucks there was no significant difference (P>0.05) across different dilutors with respect to HOS sperm percentage.

In Sirohi bucks there was significant difference between TYD and TYE as well as TYG and TYE dilutors. Significantly higher plasma membrane integrity observed in Sirohi bucks was in TYD and TYG than TYE dilutor at pre-freezing stage.

The plasma membrane integrity observed by Rasul et al. (2001) in semen of buffalo (60.4±5.6%) at equilibration period was in close agreement with present observations.

However, Salvador et al. (2006) in Murciano-Granadina bucks observed 21 to 31 % HOS positive sperm percentage in semen stored at 5°C, which was much lower than present observations. He opined that washing of semen might be affecting the plasma membrane integrity.

Fernandez-Santos et al. (2006) observed in Red Deer epididymal spermatozoa that at pre freeze stage the HOS positive sperm percentage in dilutor containing 6% glycerol was 80-85 % which was higher than present findings. This may be due to difference in the species and source of spermatozoa.
4.5.4.2 Plasma membrane integrity of spermatozoa at post-freezing (24 hours) stage (HOST).

From table 6 it is evident that the average hypo-osmotic swelling sperm percentage of semen at post-freezing (24 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 68.83±1.95, 72.66±1.78 and 68.72±1.46 and Sirohi bucks it was 68.83±0.59, 70.11±0.84 and 66.50±0.76 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks but there was a significant difference (P<0.05) across different dilutors with respect to HOS positive sperm percentage.

The results further indicated that in Osmanabadi bucks semen there was a significant difference between TYG and TYD as well as TYD and TYE dilutors. Significantly higher plasma membrane integrity was noted in TYD than TYG and TYE dilutors.

In Sirohi bucks semen there was significant difference between TYD and TYE dilutors with respect to HOS sperm percentage at post-freezing (24 hours) stage. Significantly higher plasma membrane integrity observed was in TYD than TYE dilutor. There was no significant difference between TYG and TYD as well as TYG and TYE dilutors with respect to sperm plasma membrane integrity.

The literature pertaining to post-freezing plasma membrane integrity of bucks semen was limited hence comparison was also made with other species.

Arangasamy et al. (2005) in Murrah buffalo bulls recorded 66.4 ± 0.65% HOST values for post thawed semen which was in close agreement with present observations.

Low plasma membrane integrity (less than 50 percent) was observed by Rasul et al. (2000) and Rasul et al. (2001) in post thawed buffalo bull semen, Fernandez-Santos et al. (2006) in Red Deer epididymal spermatozoa and Gang Zhang et al. (2007) in bucks from China.
Figure 8 Hypo-Osmotic Swelling Test of post thaw semen (HOST)
4.5.4.3 Plasma membrane integrity of spermatozoa at post-freezing (72 hours) stage (HOST)

From table 6 it is evident that the average hypo-osmotic swelling sperm percentage of semen at post-freezing (72 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 67.27±1.16, 70.16±1.42 and 67.83±1.23 and for Sirohi bucks it was 66.33±0.72, 67.50±1.41 and 63.66±1.10 respectively.

There was a significant difference (P<0.05) between the two breeds of bucks with respect to HOS positive sperm percentage in TYD and TYE dilutors. Plasma membrane integrity was significantly higher in Osmanabadi than Sirohi bucks in TYE dilutors at post freezing (72 hours) stage.

There was a significant difference (P<0.05) across different dilutors with respect to HOS positive sperm percentage.

The results further indicated that in Osmanabadi bucks semen there was no significant difference across different dilutors with respect to plasma membrane integrity at post freezing (72 hours) stage.

In Sirohi bucks semen there was significant difference between TYD and TYE dilutor with respect to HOS sperm percentage at post freezing (72 hours) stage. Significantly, higher percentage of plasma membrane integrity observed was in TYD than TYE dilutors.

In both the breeds higher plasma membrane integrity observed was in TYD dilutor which indicates that DMSO was more effective cryoprotectant than ethylene glycol and glycerol in preserving sperm membrane integrity on frozen storage of Osmanabadi and Sirohi bucks semen.

Arangasamy *et al.* (2005) in Murrah buffalo bulls recorded 66.4±0.65 % HOS positive sperm for post thawed semen which was in agreement with present findings.

Azerêdo *et al.* (2001) reported that in buck spermatozoa plasma membrane integrity was reduced in Tris solution, before and after freezing which was also observed in present study. The plasma membrane integrity was reduced in both Osmanabadi and Sirohi bucks semen on cryopreservation.
Rasul et al. (2000) for buffalo bull semen in citrate buffer observed lower plasma membrane integrity than present observation. They noted integrity 32.6 to 45.2 percent plasma membrane integrity as compared to 63.67 to 70.17 percent in present study. This may be due to species difference.

4.5.4.4 Plasma membrane integrity (HOST) of spermatozoa in TYG dilutor

From table 6 it is evident that the average hypo-osmotic swelling sperm percentage in TYG dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) time interval and temperatures for Osmanabadi bucks was 72.50±1.03, 68.83±1.95 and 67.27±1.16 and for Sirohi bucks it was 71.00±1.14, 68.83±0.59, and 66.33±0.72 respectively.

In TYG dilutor there was no significant difference (P>0.05) between the two breeds of bucks but there was a significant difference (P<0.05) across different time interval and temperatures with respect to hypo-osmotic swelling sperm percentage.

The results further indicated that in Osmanabadi bucks semen there was a significant difference between pre-freezing and post freezing (24 hours) as well as pre-freezing and post freezing (72 hours) stage with respect to hypo-osmotic swelling sperm percentage. Significant reduction in plasma membrane integrity was recorded at post freezing (24 and 72 hours) stage as compared to pre freezing stage.

In Sirohi bucks there was significant difference between pre-freezing and post freezing (72 hours) stage with respect to hypo-osmotic swelling sperm percentage. Significant reduction in plasma membrane integrity recorded was at post freezing (72 hours) stage as compared to pre freezing stage.

Plasma membrane integrity was not significantly reduced between 24 to 72 hours post freezing in both the breeds. This indicates that plasma membrane integrity was not damaged once the spermatozoa were frozen in TYG dilutor. It indicates that glycerol was good cryoprotectant and was able to maintain sperm membrane integrity after freezing of semen.

Rohilla et al. (2005) reported that in Murrah buffalo bull semen frozen with 6.8% glycerol the percentage of intact acrosome was 77.50±0.26% which is similar to
the present observations. Fernandez-Santos et al. (2006) recorded percentage of intact acrosomes in the semen of Red Deer which was frozen using dilutor containing glycerol. They observed that at pre freeze stage the acrosome intactness with 6% glycerol dilutor was 80-90%. In post-freezing stage it was 60-65%.

4.5.4.5 Plasma membrane integrity (HOST) of spermatozoa in TYD dilutor

From table 6 it is evident that the average hypo-osmotic swelling sperm percentage in TYD dilutors at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stage for Osmanabadi bucks was 74.22±1.02, 72.67±1.78 and 70.17±1.42 and for Sirohi bucks it was 72.11±0.89, 70.11±0.84 and 67.50±1.41 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to hypo-osmotic swelling sperm percentage at different time interval and temperatures in TYD dilutor.

There was significant difference across different time interval and temperatures with respect to hypo-osmotic swelling sperm percentage in TYD dilutor.

The results further indicated that in both the breeds there was significant difference between pre-freezing and post-freezing (24 and 72 hours) stages with respect to hypo-osmotic swelling sperm percentage in TYD dilutor. Significant reduction in hypo-osmotic swelling sperm percentage was noted between pre freezing and post freezing (24 and 72 hours) stages in TYD dilutor. But there was no significant reduction in hypo-osmotic swelling sperm percentage between post freezing 24 and 72 hours, which indicates good cryoprotecting ability of DMSO in both the breeds. Plasma membrane integrity of spermatozoa of both the breeds was maintained in TYD dilutor on frozen storage.

Leboeuf et al. (2006) had noted 36.8 to 72.9 percent membrane damaged spermatozoa by HOS test in different dilutors.
**4.5.4.6 Plasma membrane integrity (HOST) of spermatozoa in TYE dilutor**

From table 6 it is evident that average sperm plasma membrane integrity percentage detected by hypo-osmotic swelling test (HOST) in TYE dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 72.05±2.54, 68.72±1.46 and 67.83±1.23 and for Sirohi bucks it was 67.83±0.62, 66.50±0.76 and 63.66±1.10 respectively.

There was a significant difference (P<0.05) between the two breeds of bucks with respect to sperm plasma membrane integrity percentage in TYD dilutor at pre-freezing and post freezing (72 hours) stages in TYE dilutor. Significantly higher plasma membrane integrity observed was in Osmanabadi than Sirohi bucks at pre-freezing and post freezing (72 hours) stage.

There was a significant difference (P<0.05) across different time interval and temperature with respect to sperm plasma membrane integrity in TYE dilutor.

The results further indicated that in Osmanabadi bucks semen there was significant difference between pre-freezing and post-freezing (24 and 72 hours) stage with respect to sperm plasma membrane integrity detected by HOST. Significant reduction in sperm plasma membrane integrity was observed from pre-freezing to post-freezing (24 and 72 hours) stage, but plasma membrane integrity was not significantly reduced between 24 and 72 hours post freezing stage.

In Sirohi bucks semen significant difference in sperm plasma membrane integrity noted was between pre-freezing and post freezing (72 hours) stage. Plasma membrane integrity was significantly reduced from pre-freezing to post freezing (72 hours) stage, but plasma membrane integrity was not significantly reduced between 24 and 72 hours post freezing stage.

As there was no significant reduction in plasma membrane integrity from post freezing 24 to 72 hours stage indicates that plasma membrane integrity was maintained post freezing in TYE dilutor for both the breeds.

Henry *et al.* (2002) in stallion obtained post thaw-HOS positive sperm percent in five percent ethylene glycol dilutor as 14.7±11.4, which was very low as compared with present observations.

Mantovani *et al.* (2002) compared glycerol (G) and ethylene glycol (EG) as cryoprotectants for stallion semen in a skimmed milk (SM) extender. They observed
with 3% ethylene glycol post thaw HOS sperm percent was 50.9 %, which was near to the present observations.

Li et al. (2005) studied the cryoprotective effect of 11 extenders in Cynomolgus Monkey. Post thaw HOS positive sperm percent in Tris dilutor containing ethylene glycol was 54.63±7.73% which was in near agreement to the present observations.

Fernandez-Santos et al. (2006) observed that HOS sperm percentage was 60-65% and 40-50% in Red Deer epididymal spermatozoa for post-freezing with 6% glycerol and 6% ethylene glycol respectively. This indicates that HOS sperm percentage was higher when glycerol was used as cryoprotectant than ethylene glycol, which was in agreement with present findings.
### 4.5.5 Acrosome intactness of spermatozoa (Giemsa stain)

The acrosome intact sperm percentage at different freezing stages in different dilutors of Osmanabadi and Sirohi bucks detected by Giemsa stain is given in table 7.

**Table 7** Acrosome intact sperm percentage at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks (Giemsa stain)

<table>
<thead>
<tr>
<th>Dilutor</th>
<th>Acrosome intact sperm (%)</th>
<th>C.V</th>
<th>F- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-freezing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG(O)</td>
<td>Mean±S.E, n=18</td>
<td>2.779</td>
<td>3.339*</td>
</tr>
<tr>
<td>TYD(O)</td>
<td>86.89±1.00&lt;sup&gt;abc&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE(O)</td>
<td>88.50±0.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG(S)</td>
<td>85.99±0.98&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (S)</td>
<td>85.22±1.53&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>TYE(S)</td>
<td>87.38±1.44&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>85.05±1.63&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td></td>
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<tr>
<td><strong>Post-freezing 24 hr.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24hrs TYG (O)</td>
<td>85.00±1.25&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24hrs TYD (O)</td>
<td>86.94±0.94&lt;sup&gt;abc&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24hrs TYE (O)</td>
<td>84.39±0.78&lt;sup&gt;cde&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24hrs TYG (S)</td>
<td>82.89±0.73&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24hrs TYD (S)</td>
<td>86.44±1.23&lt;sup&gt;abc&lt;/sup&gt;</td>
<td></td>
<td></td>
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<tr>
<td>24hrs TYE (S)</td>
<td>83.11±1.07&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
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<tr>
<td><strong>Post-freezing 72 hr.</strong></td>
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<tr>
<td>72hrs TYG (O)</td>
<td>83.61±1.30&lt;sup&gt;de&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72 hrs TYD (O)</td>
<td>85.11±1.21&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72hrs TYE (O)</td>
<td>83.27±1.20&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72hrs TYG (S)</td>
<td>83.05±1.51&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72hrs TYD (S)</td>
<td>86.22±1.20&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72hrs TYE (S)</td>
<td>82.55±1.34&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

O=Osmanabadi S=Sirohi * Significant at 5% level. C.D (0.05) = 2.711 Means with common superscript do not differ significantly.
4.5.5.1 Acrosome intactness of spermatozoa at pre-freezing (5°C) stage

From table 7 it is evident that the average Acrosome intact sperm percentage of semen at pre-freezing (5°C) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 86.89±1.00, 88.50±0.90 and 85.99±0.98 and for Sirohi bucks it was 85.22±1.53, 87.38±1.44 and 85.05±1.63, respectively.

There was no significant difference (P>0.05) between the two breeds of bucks and across the different dilutors with respect to acrosome intact sperm percentage at pre-freezing stage (5°C).

The literature pertaining to buck semen for acrosome intactness at pre-freezing stage was limited; hence the comparison was also made with other species.

El-kon et al. (2010) recorded 91.00±0.92% intact acrosome in Damascus bucks semen after dilution was in close agreement with present observations.

The observations recorded by Fernandez-Santos et al. (2006) in Red Deer at pre freeze stage for acrosome intact sperm percentage (80-90%) for 6% glycerol dilutor was in close agreement with present findings as well as their findings for 6% ethylene glycol (70-80%) were also in agreement with present observations. This indicates that glycerol at 5% level with 5% egg yolk in tris based dilutor can maintain acrosome intactness at pre freeze stage.

The acrosome intactness observed in the range of 65 to 75 percent by following authors which was lower than present observations is as below.

Eiman and Takato (2004) in Japanese buck spermatozoa (78.0±3.3%); Sheshtawy et al. (2008) in buffalo bulls (65.30±1.73%). This could be attributed to the difference in breeds, species, dilutors, age of bucks etc.
4.5.5.2 Acrosome intactness of spermatozoa at post freezing (24 hours) stage

From table 7 it is evident that the average Acrosome intact sperm percentage of semen at post-freezing (24 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 85.00±1.25, 86.94±0.94 and 84.39±0.78 and for Sirohi bucks it was 82.89±0.73, 86.44±1.23 and 83.11±1.07 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks but there was a significant difference (P<0.05) across different dilutors with respect to acrosome intact sperm percentage with Geimsa stain.

The results further indicated that in Osmanabadi bucks semen there was no significant difference between different dilutors with respect to acrosome intact sperm percentage at post freezing (24 hours) stage.

In Sirohi bucks semen there was significant difference between TYG and TYD as well as TYD and TYE dilutors with respect to acrosome intact sperm percentage at post-freezing (24 hours) stage. Significantly higher acrosome intactness observed was in TYD dilutor in Sirohi bucks as compared to TYG and TYE dilutors. No significant difference observed across TYG and TYE dilutors. Observations recorded by Eiman and Takato (2004) regarding acrosomal intactness in Japanese bucks were similar to those recorded in present study.

Low post thaw acrosome intactness between 40 to 65 percent was observed by Chauhan et al. (1994) in Jamnapari bucks (38 to 43%), Chandler et al. (1998) in dairy bucks (46.4±1.26 to 65.5±1.26 %), Reyes et al. (2002) in boar semen (45.5 to 16.8 %), Fernandez-Santos et al. (2006) in Red Deer (6% glycerol and 6% ethylene glycol the acrosome integrity % were 60-65% and 40-50% respectively), Gang Zhang et al. (2007) in bucks China (58.42 ±18.05), Sheshtawy et al. (2008) in buffalo bulls (50.30±2), Dorado et al. (2009) in bucks (47.08± 1.43) and El-Kon et al. (2010) in Damascus bucks (54.25±0.82%) was lower than study.

Sokolovaskaya et al. (1981) reported the significance of acrosomes in semen evaluation. They suggested that thawed semen was suitable for insemination if 70 percent spermatozoa had normal acrosomes. Similarly in present study also the acrosomal damage was within normal range for both the breeds in all three diluters.
4.5.5.3 Acrosome intactness of spermatozoa at post freezing (72 hours) stage

From table 7 it is evident that the average Acrosome intact sperm percentage of semen at post-freezing (72 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 83.61±1.30, 85.11±1.21 and 83.27±1.20 and for Sirohi bucks it was 83.05±1.51, 86.22±1.20 and 82.55±1.34 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to acrosome intact sperm percent in different dilutors at post freezing (72 hours) stage.

The results further indicated that in Osmanabadi bucks semen there was no significant difference between different dilutors with respect to acrosome intact sperm percentage at post freezing (24 hours) stage.

In Sirohi bucks semen there was significant difference between TYG and TYD as well as TYD and TYE dilutors with respect to acrosome intact sperm percentage at post-freezing (24 hours) stage. Significantly higher acrosome intactness observed was in TYD dilutor in Sirohi bucks as compared to TYG and TYE dilutors. No significant difference observed across TYG and TYE dilutors.

Eiman and Takato (2004) in Japanese bucks spermatozoa noted 60 to 80 percent intact acrosome post thawing in different tris glycerol dilutors which is in agreement with present observations. Anel et al. (2003) also in ram semen observed post thaw acrosomal intactness as 74.6%, which was closer to the findings recorded in present study.

Chauhan et al. (1994) in Jamnapari bucks, Chandler et al. (1998) in dairy bucks reported higher acrosme damage in frozen semen than present observations.

4.5.5.4 Acrosome intactness of spermatozoa in TYG dilutor

From table 7 it is evident that the average acrosome intactness of spermatozoa in TYG dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stage for Osmanabadi bucks was 86.89±1.00, 85.00±1.48 and
83.61±1.30 and for Sirohi bucks it was 85.22±1.53, 82.89±0.86 and 83.05±1.51 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to acrosome intact sperm percentage across different time interval and temperature in TYE dilutor.

There was significant difference (P>0.05) across different time interval and temperatures with respect to acrosome intactness in TYG dilutor.

The result further indicated that in Osmanabadi bucks semen there was significant reduction in acrosome intact sperm percent from pre freezing to post freezing (72 hours) stage, but there was no significant reduction in acrosome intactness observed between post freezing 24 and 72 hours stage in TYG dilutor.

In Sirohi bucks semen there was no significant difference across different time interval and temperature with respect to acrosome intact sperm percent in TYG dilutor. In both the breeds as there was no significant reduction in acrosome intactness between post freezing 24 and 72 hours stage it can be concluded that glycerol was effective in maintaining acrosome intactness of bucks spermatozoa.

Eiman and Takato (2004) observed that addition of glycerol as cryoprotectant improved the sperm acrosomal intactness. They observed 80 % intact acrosome in Tris citric acid glucose- egg yolk +glycerol dilutor. This is in close agreement with present observations.

4.5.5.5 Acrosome intactness of spermatozoa in TYD dilutor

From table 7 it is evident that the average acrosome intactness of spermatozoa detected by Geimsa stain in TYD dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stage for Osmanabadi bucks was 88.50±0.90, 86.94±1.12 and 85.11±1.21 % and for Sirohi bucks it was 87.38±1.44, 86.44±1.46 and 86.22 ± 1.20 % respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to acrosome intact sperm percentage across different time interval and temperature in TYD dilutor.
There was significant difference (P<0.05) across different time interval and temperatures with respect to acrosome intactness in TYD dilutor.

The result further indicated that in Osmanabadi bucks semen there was significant reduction in acrosome intact sperm percent from pre freezing to post freezing (72 hours) stage, but there was no significant reduction in acrosome intactness observed between post freezing 24 and 72 hours stage in TYD dilutor.

In Sirohi bucks semen there was no significant difference across different time interval and temperature with respect to acrosome intact sperm percent in TYD dilutor. In both the breeds as there was no significant reduction in acrosome intactness between post freezing 24 and 72 hours stage it can be concluded that DMSO was effective in maintaining acrosome intactness of bucks spermatozoa.

On scanning the available literature, the references regarding acrosomal intact sperm percentage of semen of Osmanabadi and Sirohi bucks in TYD dilutor at different time interval and temperatures were not available. Therefore no comparison could be made.

4.5.5.6 Acrosome intactness of spermatozoa in TYE dilutor

From table 7 it is evident that the average acrosome intactness of spermatozoa by Geimsa stain in TYE dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stage for Osmanabadi bucks was 85.99±0.98, 84.39±0.92, 83.27±1.20 % and for Sirohi bucks it was 85.05±1.63, 83.11±1.26, 82.55±1.34% respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to acrosome intact sperm percentage across different time interval and temperature in TYE dilutor.

There was significant difference (P<0.05) across different time interval and temperatures with respect to acrosome intactness in TYE dilutor.

The result further indicated that in Osmanabadi bucks semen there was significant reduction in acrosome intact sperm percent from pre freezing to post freezing (72 hours) stage, but there was no significant reduction in acrosome intactness observed between post freezing 24 and 72 hours stage in TYE dilutor.
In Sirohi bucks semen there was no significant difference across different time interval and temperature with respect to acrosome intact sperm percent in TYE dilutor. In both the breeds as there was no significant reduction in acrosome intactness between post freezing 24 and 72 hours stage it can be concluded that ethylene glycol was effective in maintaining acrosome intactness of bucks spermatozoa.

Li et al. (2005) studied cryoprotective effect of 11 extenders in Cynomolgus Monkey. Post thaw acrosome intact sperm percent was 81.92±5.87 in Tris dilutor containing ethylene glycol, which was in close agreement with present observations.

Rohilla et al. (2005) in Murrah buffalo bull semen freezing used 6.8% glycerol, 5% ethylene glycol and 3% propylene glycol as cryo-protectant. Semen frozen in 6.8% glycerol resulted higher intact acrosome (77.50±0.26) followed by semen extended with 5% ethylene glycol (73.70±0.34). This is in agreement with present observations as we had also found higher acrosome intact sperm percent in TYG than TYE dilutor for both the breeds of bucks.

Martins-Bessa et al. (2006) observed in dogs that the acrosome intact sperm percent in dilutor with 5% ethylene glycol was 37.8±11.0 and dilutor with 4% ethylene glycol was 36.1±11.9. These observations were lower than present findings.

Fernandez-Santos et al.(2006) studied that in Red Deer at post-freezing for 6% glycerol and 6% ethylene glycol the acrosome integrity percent was 60-65% and 40-50% respectively. Same trend was observed in present study where we had observed higher acrosome intact sperm percent in TYG dilutor than TYE dilutor.
4.6.6 DNA damaged of spermatozoa (Acridine Orange test)

The DNA damaged sperm percentage at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks detected by Acridine Orange test is given in table no 8.

Table 8 DNA damaged of sperm percentage at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks (Acridine Orange test)

<table>
<thead>
<tr>
<th>Stage of freezing</th>
<th>DNA damaged sperm %</th>
<th>C.V</th>
<th>F- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-freezing.</td>
<td>Mean±S.E, n=18</td>
<td>41.952</td>
<td>20.081*</td>
</tr>
<tr>
<td>TYG(O)</td>
<td>00.00 f</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD(O)</td>
<td>00.00 f</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE(O)</td>
<td>00.00 f</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG(S)</td>
<td>00.00 f</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD(S)</td>
<td>00.00 f</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE(S)</td>
<td>00.00 f</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-freezing 24 hr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG (O)</td>
<td>5.66±0.95 CDE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD(O)</td>
<td>4.83±1.02 e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE (O)</td>
<td>8.15±0.85 AB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG (S)</td>
<td>6.55±0.74 BCDE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (S)</td>
<td>5.41±1.40 E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE (S)</td>
<td>5.77±0.55 CDE</td>
<td></td>
<td></td>
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<tr>
<td>Post-freezing 72 hr.</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TYG (O)</td>
<td>6.44±1.05 BCDE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (O)</td>
<td>6.38±1.01 BCDE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE (O)</td>
<td>8.94±0.58 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG (S)</td>
<td>8.94±0.82 ABC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (S)</td>
<td>7.77±1.76 ABCDE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE (S)</td>
<td>7.89±0.70 a</td>
<td></td>
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</tbody>
</table>

O=Osmanabadi S= Sirohi. *Significant at 5% level. C.D. (0.05) =2.217. Means with common superscript do not differ significantly.
Figure. 10 Acridin orange test for DNA damage in neat semen

Figure. 11 Acridin orange test for DNA damage in post thaw semen
4.5.6.1 DNA damaged spermatozoa at pre-freezing (5°C) stage (Acridine Orange test).

From table 8 it is evident that there was no DNA damaged sperm detected by Acridine orange test at pre-freezing (5°C) stage in TYG, TYD and TYE dilutors for Osmanabadi and Sirohi bucks.

Martins et al. (2007) recorded only 0.5% DNA damage in cooled semen in Nellore bulls which was close to present observations.

4.5.6.2 DNA damaged spermatozoa at post-freezing (24 hours) stage (Acridine Orange test).

From table 8 it is evident that average DNA damaged sperm percentage detected by Acridine orange test in semen at post-freezing (24 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 5.66±0.95, 4.83±1.02 and 8.15±0.85 and for Sirohi bucks it was 6.55± 0.74, 5.41± 1.40 and 5.77±0.55 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks in TYG and TYD dilutors with respect to DNA damaged sperm percent but there was significantly higher (P<0.05) DNA damaged sperm detected in Osmanabadi than Sirohi bucks in TYE dilutor at post freezing (24 hours) stage.

There was significant difference between DNA damaged sperm percent across different dilutors at post freezing (24 hours) stage.

The results further indicated that in Osmanabadi bucks semen there was significant difference between TYG and TYE as well as TYD and TYE dilutors with respect to DNA damaged sperm percent at post freezing (24 hours) stage. Significantly higher DNA damaged sperm percentage observed was in TYE than TYG and TYD dilutors. No significant difference was observed between TYG and TYD dilutors with respect to DNA damaged sperm percent at post freezing (24 hours) stage.

In Sirohi bucks semen there was no significant difference observed across different dilutors at post freezing (24 hours) stage with respect to DNA damaged sperm percent.
DNA damaged sperm in the range of 5 to 10 percent observed by Thuwanut et al. (2007) in post thawed semen in cats was in agreement with present observations; however very high DNA damaged sperm percent was noted by Chohan et al. (2004) in human post thawed semen than present observations.

4.5.6.3 DNA damaged spermatozoa at post-freezing (72 hours) stage (Acridine Orange test).

From table 8 it is evident that the average DNA damaged sperm percentage detected by Acridine orange test in semen at post-freezing (72 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 6.44±1.05, 6.38±1.01 and 8.94±0.58 and for Sirohi bucks it was 7.89±0.82, 7.77±1.76 and 8.94±0.70 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to DNA damaged sperm percent at post freezing (72 hours) stage in different dilutors.

There was significant difference between DNA damaged sperm percent across different dilutors at post freezing (72 hours) stage.

The results further indicated that in Osmanabadi bucks semen there was significant difference between TYG and TYE as well as TYD and TYE dilutors with respect to DNA damaged sperm percent at post freezing (72 hours) stage. Significantly higher DNA damaged sperm percentage observed was in TYE than TYG and TYD dilutors. There was no significant difference between TYG and TYD dilutors with respect to DNA damaged sperm percent at post freezing (72 hours) stage.

In Sirohi bucks semen there was no significant difference observed across different dilutors at post freezing (72 hours) stage with respect to DNA damaged sperm percent.

Ali et al. (2008) opined that there was no significant difference in the percentage of spermatozoa with intact DNA between the frozen semen of bucks in different extenders which was in agreement with present observations. In present study also on significant DNA damage was observed across the three dilutors.
4.5.6.4 DNA damaged spermatozoa in TYG dilutor (Acridine Orange test)

From table 8 it is evident that the average DNA damaged spermatozoa percentage detected by Acridine Orange (AO) test in TYG dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 0.00, 5.66±1.12, and 6.44±1.05 and for Sirohi bucks it was 0.00, 6.55±0.88 and 7.89±0.70 respectively.

There was no significant difference (P>0.05) observed at pre-freezing, 24 and 72 hours post-freezing stages with respect to DNA damaged sperm percentage detected by AO test in TYG dilutor. DNA damaged sperm were detected only after freezing in TYG dilutor, it indicates that freezing causes DNA damage in sperm.

The DNA damaged sperm were detected after freezing in TYG dilutor but there was no significant increase in DNA damaged sperm percentage from 24 to 72 hours post freezing in both the breeds, it indicates that sperm DNA damage was not increased significantly after freezing in TYG dilutor.

On scanning available literature, references regarding post-freezing DNA damage of spermatozoa especially in TYG dilutor was not found so comparison could not be made.

4.5.6.5 DNA damaged spermatozoa in TYD dilutor (Acridine Orange test)

From table 8 it is evident that the average DNA damaged sperm percentage detected by Acridine orange (AO) test in TYD dilutors at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 0.00, 4.83±1.21 and 6.38±1.01 and for Sirohi bucks it was 0.00, 5.41±1.65 and 7.77±1.76 respectively.

There was no significant difference (P>0.05) observed at pre-freezing, 24 and 72 hours post-freezing stages with respect to DNA damaged sperm percentage detected by AO test in TYD dilutor. DNA damaged sperm were detected only after freezing in TYD dilutor, it indicates that freezing causes DNA damage in sperm.
The DNA damaged sperm were detected after freezing in TYD dilutor but there was no significant increase in DNA damaged sperm percentage from 24 to 72 hours post freezing stage in both the breeds, it indicates that sperm DNA damage was not increased significantly after freezing in TYD dilutor.

On scanning the available literature, the references regarding DNA damaged sperm percentage in semen of Osmanabadi and Sirohi bucks in TYD dilutor at different time interval and temperatures were not available. Therefore no comparison could be made.

4.5.6.6 DNA damaged spermatozoa in TYE dilutor (Acridine Orange test)

From table 8 that the average DNA damaged sperm percentage detected by Acridine orange (AO) test in TYE dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 00.00, 8.15 ± 0.85 and 8.94 ± 0.58 % and for Sirohi bucks it was 00.00, 5.77 ± 0.55 and 7.89 ± 0.70 % respectively.

There was significant difference (P<0.05) between the two breeds of bucks at post freezing (24 hours) stage with respect to DNA damaged sperm percent in TYE dilutor. Significantly higher DNA damaged sperm were detected at post freezing (24 hours) stage in Osmanabadi than Sirohi bucks.

The result further suggested that in Osmanabadi bucks semen there was no significant difference between post freezing 24 and 72 hours stages with respect to DNA damaged sperm percent in TYE dilutor, but in Sirohi bucks semen there was significant difference between 24 and 72 hours post freezing stage. Significantly higher DNA damaged sperm were detected at 72 hrs than 24 hrs post freezing stage in TYE dilutor in Sirohi bucks; which indicates DNA damage was increasing as time progressed post freezing in TYE dilutor which could be the reason of low fertility.

On scanning the available literature, the references regarding DNA damaged sperm percentage post freezing in semen of Osmanabadi and Sirohi bucks in TYD dilutor at different time interval and temperatures were not available. Therefore no comparison could be made.
4.5.7 DNA damaged spermatozoa percentage (Comet assay)

The DNA damaged spermatozoa percentage at different freezing stages indifferent dilutors in Osmanabadi and Sirohi bucks semen detected by Comet assay is given in table 9.

Table 9 DNA damaged spermatozoa percentage at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks (Comet assay)

<table>
<thead>
<tr>
<th>Stage of freezing</th>
<th>DNA damaged sperm %</th>
<th>C.V</th>
<th>F-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-freezing</td>
<td>Mean±S.E, n=18</td>
<td>50.845</td>
<td>13.098*</td>
</tr>
<tr>
<td>TYG(O)</td>
<td>0.00e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD(O)</td>
<td>0.00e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE(O)</td>
<td>0.00e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG(S)</td>
<td>0.00e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (S)</td>
<td>0.00e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE (S)</td>
<td>0.00e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-freezing 24 hr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG (O)</td>
<td>1.49±0.18bcd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (O)</td>
<td>1.33±0.30bcd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE (O)</td>
<td>1.28±0.16cde</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG (S)</td>
<td>1.38±0.31bcd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (S)</td>
<td>1.15±0.26d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE (S)</td>
<td>1.43±0.18bcd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-freezing 72 hr</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TYG (O)</td>
<td>1.89±0.20abc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (O)</td>
<td>1.66±0.31bcd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE (O)</td>
<td>1.83±0.20abc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG (S)</td>
<td>1.94±0.29ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (S)</td>
<td>1.83±0.27abc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE (S)</td>
<td>2.44±0.31a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

O=Osmanabadi S=Sirohi, * significant at 5% level, C.D. (0.05) =0.645
Means with common superscript do not differ significantly.
4.5.7.1 DNA damaged spermatozoa at pre-freezing (5°C) stage (Comet assay)

From table 9 it is evident that there was no DNA damage detected by Comet assay at pre-freezing (5°C) stage in TYG, TYD and TYE dilutors for Osmanabadi and Sirohi bucks.

The DNA damaged sperm percentages reported by Fraser and Strzezek (2004) in boar (2.90±0.28, 3.05±0.32 and 2.95±0.30 percent), Boe-Hansen et al. (2005) in bulls (1.49 to 3.26 percent) and Nandre (2007) in buffalo bull (6.97±1.13%) at pre-freezing stage were higher than present observations. This could be attributed to dilutor used and species variations. In present study it was observed that addition of dilutors to semen did not affect the DNA at pre-freezing stage.

4.5.7.2 DNA damaged spermatozoa at post-freezing (24 hours) stage (Comet assay)

From table 9 it is evident that the average DNA damage sperm percentage detected by Comet assay in semen at post-freezing (24 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 1.49±0.18, 1.38±0.30 and 1.28±0.16 and for Sirohi bucks it was 1.38±0.31, 1.15±0.26 and 1.43±0.18 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks as well as across different dilutors with respect to DNA damaged sperm percentage detected by Comet assay at post-freezing (24 hours) stage.

Fraser et al. (2006) in bulls reported 1% DNA damaged sperm, which is similar to present observations. But Donnelly et al. (2001) in human (16.83%) Isachenko et al. (2004) in human (9%), Nandre (2007) in buffalo bull spermatozoa (9.11±1.31%) and Berlinguer et al. (2009) in adult male goats (10.2 ± 1.8 to 14.9 ± 1.8) reported higher DNA damaged sperm percent than present observations. This higher DNA damage could be due to different protocol of cryopreservation, different dilutors used, season of semen collection etc.
Figure 12 DNA damaged test for post thaw semen (Comet assay)
4.5.7.3 DNA damaged spermatozoa at post-freezing(72 hours) stage (Comet assay)

From table 9 it is evident that the average DNA damaged sperm percentage detected by Comet assay in semen at post-freezing (72 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 1.89 ± 0.20, 1.66±0.31, and 1.83 ± 0.20 and for Sirohi bucks it was 1.94 ± 0.29, 1.83±0.27 and 2.44±0.31 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks as well as across different dilutors with respect to DNA damage detected by Comet assay at post freezing (72 hours) stage.

Berlinguer et al. (2009) observed 10.2 ± 1.8 to 14.9 ± 1.8 percent DNA damage by Comet assay in frozen/thawed spermatozoa collected from three adult male goats. These observations are higher than present findings. Nandre (2007) also observed higher (11 to 13 %) DNA damage in buffalo bull spermatozoa on cryopreservation than present observations which could be due to difference in species and season of semen collection.

4.5.7.4 DNA damaged spermatozoa in TYG dilutor (Comet assay)

From table 9 it is evident that the average DNA damage of spermatozoa detected by Comet assay in TYG dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 00.00, 1.49 ± 0.18 and 1.89 ± 0.20 and for Sirohi bucks it was 00.00, 1.38 ± 0.36 and 1.94 ± 0.29 respectively.

There was no significant difference (P>0.05) observed in the two breeds of bucks as well as at 24 and 72 hours post-freezing stage with respect to DNA damaged sperm percentage detected by Comet assay in TYG dilutor. DNA damaged sperm noted only after freezing in TYG dilutor, but the damage was not significantly increased at 72 hours post-freezing.
Fraser et al. (2006) reported about 1% DNA damage on freezing of bull spermatozoa which was in close agreement with present observations where about 1% DNA damage in the both the breeds of bucks was observed on preservation of semen in TYG dilutor. But Li et al. (2007) reported 0 to 2.7% DNA damage in monkey spermatozoa after freezing, which increased to 52.7 to 92.0% after thawing. These observations are not in agreement with present findings which may be due to difference of species, cryoprotectant used and its concentration etc.

4.5.7.5 DNA damaged spermatozoa in TYD dilutor (Comet assay)

From table 9 it is evident that percent DNA damaged spermatozoa detected by comet assay in TYD dilutors at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stage for Osmanabadi bucks was 0.00, 1.38±0.36, 1.66±0.31 and for Sirohi bucks it was 0.00, 1.15±0.31, 1.83±0.27 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks as well as there was no significant difference (P>0.05) across post freezing (24 and 72 hours) stages with respect to percent DNA damaged spermatozoa detected by Comet assay in TYD dilutor. This indicates that however in TYD dilutor there was damage to sperm DNA on freezing it was not significantly increased after freezing.

On scanning the available literature, the references regarding DNA damaged sperm percentage of semen of Osmanabadi and Sirohi bucks in TYD dilutor at different time interval and temperatures were not available. Therefore no comparison could be made.

4.5.7.6 DNA damaged spermatozoa in TYE dilutor (Comet assay)

From table 9 it is evident that average DNA damaged spermatozoa percent detected by comet assay in TYE dilutors at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 0.00,
1.28±0.20 and 1.83±0.20 and for Sirohi bucks it was 0.00, 1.43±0.20 and 2.44±0.31 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks at pre-freezing and post freezing (24 and 72 hours) stages.

There was significant difference (P<0.05) across different time interval and temperatures with respect to DNA damaged spermatozoa detected by comet assay in TYE dilutor.

The results further indicated that in Osmanabadi bucks there was no significant difference between post-freezing (24 hours) and post freezing (72 hours) stage with respect to DNA damaged sperm percentage, which indicates DNA damage was not increasing as time progressed post freezing in TYE dilutor.

In Sirohi bucks there was significant difference between post freezing (24 hours) and post freezing (72 hours) stage with respect to DNA damaged sperm percentage. Significantly higher DNA damaged sperm percentage recoded was at post freezing (72 hours) than post-freezing (24 hours) stage; which indicates DNA damage was increasing as time progressed post freezing in TYE dilutor which could be reason of low fertility.

On scanning the available literature, the references regarding DNA damage sperm percentage post freezing in semen of Osmanabadi and Sirohi bucks in TYE dilutor at different time interval and temperatures were not available; therefore, comparison could not be made.
4.5.8 Glutamic – Oxaloacetic Transaminase (GOT) and Glutamic – Pyruvate Transaminase (GPT) activity

The Glutamic – Oxaloacetic Transaminase (GOT) activity at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks semen is given in table 10.

Table 10 Transaminase (GOT and GPTIU/L) activity at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks semen.

<table>
<thead>
<tr>
<th>Dilutor</th>
<th>GOT (IU/L)</th>
<th>C.V</th>
<th>F-Value</th>
<th>GPT (IU/L)</th>
<th>C.V</th>
<th>F-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-freezing</td>
<td>Mean±S.E, n=18</td>
<td>13.211</td>
<td>4.021*</td>
<td>Mean±S.E, n=18</td>
<td>8.745</td>
<td>12.666</td>
</tr>
<tr>
<td>TYG(O)</td>
<td>110.30±8.69&lt;sup&gt;abcde&lt;/sup&gt;</td>
<td>20.06±1.12&lt;sup&gt;h&lt;/sup&gt;</td>
<td>19.49±1.08&lt;sup&gt;h&lt;/sup&gt;</td>
<td>22.72±1.11&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>20.11±0.89&lt;sup&gt;h&lt;/sup&gt;</td>
<td>20.38±0.94&lt;sup&gt;ghi&lt;/sup&gt;</td>
</tr>
<tr>
<td>TYD(O)</td>
<td>101.33±7.47&lt;sup&gt;defghi&lt;/sup&gt;</td>
<td>20.00±0.98&lt;sup&gt;ghi&lt;/sup&gt;</td>
<td>21.72±0.94&lt;sup&gt;ghi&lt;/sup&gt;</td>
<td>22.89±1.08&lt;sup&gt;ghi&lt;/sup&gt;</td>
<td>20.11±0.89&lt;sup&gt;h&lt;/sup&gt;</td>
<td>20.38±0.94&lt;sup&gt;ghi&lt;/sup&gt;</td>
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<tr>
<td>TYE(O)</td>
<td>112.22±8.01&lt;sup&gt;abcdef&lt;/sup&gt;</td>
<td>24.05±1.22&lt;sup&gt;defghi&lt;/sup&gt;</td>
<td>23.50±1.04&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>22.89±1.24&lt;sup&gt;def&lt;/sup&gt;</td>
<td>21.72±1.03&lt;sup&gt;ghi&lt;/sup&gt;</td>
<td>20.00±1.12&lt;sup&gt;ghi&lt;/sup&gt;</td>
</tr>
<tr>
<td>TYG(S)</td>
<td>87.55±3.50&lt;sup&gt;ghi&lt;/sup&gt;</td>
<td>24.16±0.80&lt;sup&gt;defghi&lt;/sup&gt;</td>
<td>24.16±0.80&lt;sup&gt;defghi&lt;/sup&gt;</td>
<td>24.16±0.80&lt;sup&gt;defghi&lt;/sup&gt;</td>
<td>24.16±0.80&lt;sup&gt;defghi&lt;/sup&gt;</td>
<td>24.16±0.80&lt;sup&gt;defghi&lt;/sup&gt;</td>
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<tr>
<td>TYD (S)</td>
<td>86.16±3.50&lt;sup&gt;ghi&lt;/sup&gt;</td>
<td>24.77±0.84&lt;sup&gt;defghi&lt;/sup&gt;</td>
<td>24.77±0.84&lt;sup&gt;defghi&lt;/sup&gt;</td>
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<td>24.77±0.84&lt;sup&gt;defghi&lt;/sup&gt;</td>
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<tr>
<td>TYE (S)</td>
<td>94.05±4.91&lt;sup&gt;ghi&lt;/sup&gt;</td>
<td>28.33±0.70&lt;sup&gt;abcdef&lt;/sup&gt;</td>
<td>28.33±0.70&lt;sup&gt;abcdef&lt;/sup&gt;</td>
<td>28.33±0.70&lt;sup&gt;abcdef&lt;/sup&gt;</td>
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<td>28.33±0.70&lt;sup&gt;abcdef&lt;/sup&gt;</td>
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<tr>
<td>Post-freezing 24hr.</td>
<td>Mean±S.E, n=18</td>
<td>22.66±1.12&lt;sup&gt;efghi&lt;/sup&gt;</td>
<td>22.66±1.12&lt;sup&gt;efghi&lt;/sup&gt;</td>
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<td>22.66±1.12&lt;sup&gt;efghi&lt;/sup&gt;</td>
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<tr>
<td>TYG (O)</td>
<td>116.33±8.16&lt;sup&gt;abcdef&lt;/sup&gt;</td>
<td>25.05±0.65&lt;sup&gt;defghi&lt;/sup&gt;</td>
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<td>25.05±0.65&lt;sup&gt;defghi&lt;/sup&gt;</td>
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<td>TYD (O)</td>
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<td>24.16±0.80&lt;sup&gt;defghi&lt;/sup&gt;</td>
<td>24.16±0.80&lt;sup&gt;defghi&lt;/sup&gt;</td>
<td>24.16±0.80&lt;sup&gt;defghi&lt;/sup&gt;</td>
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<td>TYE (O)</td>
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<td>24.77±0.84&lt;sup&gt;defghi&lt;/sup&gt;</td>
<td>24.77±0.84&lt;sup&gt;defghi&lt;/sup&gt;</td>
<td>24.77±0.84&lt;sup&gt;defghi&lt;/sup&gt;</td>
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<td>24.77±0.84&lt;sup&gt;defghi&lt;/sup&gt;</td>
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<tr>
<td>TYG (S)</td>
<td>98.44±5.22&lt;sup&gt;defghi&lt;/sup&gt;</td>
<td>28.33±0.70&lt;sup&gt;abcdef&lt;/sup&gt;</td>
<td>28.33±0.70&lt;sup&gt;abcdef&lt;/sup&gt;</td>
<td>28.33±0.70&lt;sup&gt;abcdef&lt;/sup&gt;</td>
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<td>TYD (S)</td>
<td>91.94±4.42&lt;sup&gt;ghi&lt;/sup&gt;</td>
<td>30.50±0.69&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>30.50±0.69&lt;sup&gt;abc&lt;/sup&gt;</td>
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<td>30.50±0.69&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
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<td>TYE (S)</td>
<td>106.22±5.43&lt;sup&gt;bcdefg&lt;/sup&gt;</td>
<td>33.33±0.91&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>33.33±0.91&lt;sup&gt;abcd&lt;/sup&gt;</td>
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</tr>
</tbody>
</table>

O=Osmanabadi S=Sirohi, *significant at 5% level. Means with common superscript do not differ significantly. GOT C.D (0.05) =16.115, GPT C.D (0.05) = 2.399
4.5.8.1 GOT and GPT at pre-freezing (5°C) stage.

From table 10 it is evident that at pre-freezing (5°C) stage average GOT in TYG, TYD and TYE dilutors for Osmanabadi bucks was 110.30±8.69, 101.33±7.47, 112.22±8.01 IU/L and for Sirohi bucks it was 87.55±3.50, 86.16±3.50 and 94.05±4.91 IU/L respectively.

There was significant difference (P<0.05) between the two breeds of bucks with respect to GOT leakage except TYD dilutor. Significantly higher leakage of GOT was noted in TYG and TYE dilutors in Osmanabadi than Sirohi bucks.

There was no significant difference (P>0.05) across different dilutors with respect to GOT in semen of Osmanabadi and Sirohi bucks at pre-freezing stage. GOT release was not differing significantly in all three dilutors indicates that change in dilutors did not affect the GOT at pre-freeze stage of semen.

From table 10 it is evident that at pre-freezing (5°C) stage the average GPT in TYG, TYD and TYE dilutors for Osmanabadi bucks was 20.06±1.12, 19.49±1.08 and 22.72±1.11 IU/L and for Sirohi bucks it was 20.11±0.89, 20.38±0.94 and 24.05±1.22 IU/L respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to GPT. GPT was not affected by breed difference.

There was significant difference (P<0.05) across different dilutors with respect to GPT.

The results further indicated that in both the breeds there was a significant difference between dilutors TYG-TYE, and TYD- TYE with respect to GPT in both the breeds. GPT leakage was higher in TYE than TYG and TYD dilutor. There was no significant difference in GPT leakage in TYG and TYD dilutor. This indicates that sperm damage was higher in TYE than other two dilutors.

In present study there was higher GOT release than GPT in both the breeds of bucks semen at pre-freezing stage. Similar type of trend was observed by Mohan and Razdan (1982) in buffalo semen in 3 different diluents, Dhami et al. (1990) in Surti buffalo bulls, Singh et al. (1991) in Murrah buffalo bulls and Chauhan et al. (1994) in Jamnapari bucks.
4.5.8.2 GOT and GPT at post-freezing (24 hours) stage.

From table 10 it is evident that at post-freezing (24 hours) stage the average GOT in TYG, TYD and TYE dilutors for Osmanabadi bucks was 116.33±8.16, 108.11±7.94 and 118.00±7.46 IU/L and for Sirohi bucks it was 98.44±5.22, 91.94±4.42 and 106.22±5.43 IU/L respectively.

There was a significant difference (P<0.05) between the two breeds of bucks with respect to GOT leakage in TYG and TYE dilutors. Significantly, higher GOT leakage noted was in TYG and TYE dilutors in Osmanabadi than Sirohi bucks at post-freezing (24 hours) stage. This indicates higher sperm damage in Osmanabadi bucks than Sirohi bucks.

There was no significant difference (P>0.05) across different dilutors with respect to GOT at post-freezing (24 hours) stage. This indicates that all three dilutors were providing optimum condition for spermatozoa with respect to GOT levels.

From table 14 it is evident that at post-freezing (24 hours) stage the average GPT in TYG, TYD and TYE dilutors for Osmanabadi bucks was 22.66±1.12, 21.72±1.03 and 25.05±0.65 IU/L and for Sirohi bucks it was 24.16±0.80, 24.77±0.84 and 28.33±0.70 IU/L respectively.

There was a significant difference (P<0.05) between the two breeds of bucks with respect to GPT in TYD and TYE dilutors. Significantly higher GPT was recorded in Sirohi than Osmanabadi bucks semen in TYD and TYE dilutors at post-freezing (24 and 72 hours) stage.

There was significant difference (P<0.05) across different dilutors with respect to GPT at post-freezing (24 hours) stage.

In Osmanabadi and Sirohi bucks there was significant difference in TYD and TYE dilutors with respect to GPT at post-freezing (24 hours) stage. Significantly higher GPT leakage noted was in TYE than TYD dilutor at post freezing (24 hours) stage in both the breeds. In Osmanabadi bucks no significant difference observed between TYG and TYD as well as TYG and TYE dilutor. In Sirohi bucks semen significant difference was also noted in TYG and TYE dilutors. Significantly higher GPT was noted in TYE than TYG dilutor indicates higher sperm damage in TYE than TYG dilutor.

The literature pertaining to transaminase leakage especially post-freezing for buck semen was limited; hence comparison with other species was also made.
In present study there was higher GOT release than GPT in both the breed of
bucks at 24 hours post-freezing stage. Similar trend was observed by Tuli et al.
(1991) in Murrah buffalo bulls.

Tuli and Holtz (1992) in Boer bucks and Chauhan et al. (1994) in Jamanpari
bucks used GOT release to evaluate semen before and after semen preservation
which is in agreement with present study.

**4.5.8.3 GOT and GPT at post-freezing (72 hours) stage.**

From table 10 it is evident that at post-freezing (72 hours) stage average
GOT in TYG, TYD and TYE dilutors for Osmanabadi bucks was 119.72±7.75,
112.50±7.54 and 123.33±7.12 IU/L and for Sirohi bucks it was 102.50±4.96,
103.06±5.51 and 121.22±7.25 IU/L respectively.

At post-freezing (72 hours) stage there was no significant difference (P>0.05)
between the two breeds of bucks in TYD and TYE dilutor, but there was significant
difference (P<0.05) between two breeds of bucks in TYG dilutor. Significantly higher
GOT was recorded in Osmanabadi than Sirohi bucks in TYG dilutor at post freezing
(72 hours) stage.

The result further also indicated that there was significant leakage of GOT in
both the breeds in TYG and TYE as well TYD and TYE dilutors. Significantly higher
GOT was noted in TYE dilutor as compared to TYG and TYD dilutors at post freezing
(72 hours) stage. There was no significant difference between TYG and TYD dilutor
with respect to GOT in both the breeds

From table 14 it is evident that at post-freezing (72 hours) stage GPT in TYG,
TYD and TYE dilutors for Osmanabadi bucks was 23.50±1.04, 22.89±1.24 and
26.33±0.81 IU/L and for Sirohi bucks it was 26.11±0.63, 27.16±0.91, 30.50±0.69 IU/L
respectively.
There was a significant difference (P<0.05) between the two breeds of bucks with respect to GPT in all three dilutors at post freezing (72 hours) stage. GPT leakage was more in Sirohi than Osmanabadi bucks.

There was a significant difference (P<0.05) across different dilutors with respect to GPT at post-freezing (72 hours) stage.

The results further indicted that in both the breeds there was a significant difference between TYG and TYE as well as TYD and TYE dilutors with respect to GOT at post freezing (72 hours) stage. Significantly, higher GPT leakage noted was in TYE than TYD and TYG dilutors.

There was no significant difference between TYG and TYD dilutor in both the breeds of bucks with respect to GPT at post freezing (72 hours) stage.

Significantly, higher GPT leakage noted was in TYE than TYD and TYG dilutors in both the breeds indicates, that DMSO was better cryoprotectant than ethylene glycol and glycerol for semen preservation of both breeds.

Singh et al. (1991) studied total 40 ejaculates from five Murrah buffalo bulls at weekly interval. The overall GOT (n mol pyruvate formed/min/ml) in Tris yolk glycerol after 7 days post-freezing was 1250.8±68.11 whereas GPT leakage was 138.64 ± 10.91. Similar trend of higher GOT leakage than GPT was observed in present study. Tuli and Holtz (1992) recorded leakage of GOT (132 to 137 U/Ml) in Boer bucks semen at post-freezing stage (7 days), similar observations also recorded in present study. There was leakage of GOT in all three dilutors between pre freeze and post freeze stage of semen preservation for both the breeds of bucks.

4.5.8.4 GOT and GPT in TYG dilutor.

From table 10 it is evident that the average GOT in TYG dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 110.30±8.69, 116.33±8.16 and 119.72±7.75 IU/L and for Sirohi bucks it was 87.55±3.50, 98.44±5.22 and 102.50±4.96 IU/l respectively.

In TYG dilutor there was a significant difference (P<0.05) between the two breeds of bucks. Significantly higher GOT leakage observed was in Osmanabadi than Sirohi bucks at all freezing stages in TYG dilutor.
There was no significant difference (P>0.05) across different time interval and temperatures with respect to GOT in TYG dilutor which indicates that GOT leakage was not significantly increased in TYG dilutor from pre-freezing to post-freezing.

From table 14 it is evident that the average GPT in TYG dilutor at pre-freezing (5°C), post freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 20.06±1.12, 22.66±1.12, and 23.50±1.04 IU/l and Sirohi bucks it was 20.11±0.89, 24.16±0.80 and 26.11±0.63 IU/L respectively.

There was no significant difference (P>0.05) between the two breeds of bucks at Pre-freezing and post freezing (24 hours) stage but there was significant difference at post freezing (72 hours) stage with respect to GPT in TYG dilutor. Significantly, higher GPT observed was in Sirohi than Osmanabadi bucks at post freezing (72 hours) stage.

There was a significant difference (P<0.05) across different time interval and temperatures with respect to GPT in TYG dilutor.

The results further indicated that in both breeds there was a significant difference between pre-freezing and 24 hours post-freezing as well as pre-freezing and 72 hours post-freezing stages in TYG dilutor with respect to GPT. There was no significant difference between 24 and 72 hours post freezing stages with respect to GPT in TYG dilutor. This indicates significant higher leakage of GPT immediately after freezing, but leakage reduced subsequently in TYG dilutor.

Tuli et al. (1986) in Murraha buffalo bulls, Dhami and Kodagali (1990) in Surti buffalo bulls and Singh et al. (1991) in Murrah buffalo bulls observed similar trend of GOT and GPT leakage as observed in present study.

Tuli and Holtz (1992) also observed leakage of GPT after freezing the Boer buck semen.

### 4.5.8.5 GOT and GPT in TYD dilutor

From table 10 it is evident that GOT in TYD dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 101.33±7.47, 108.11±7.94 and 112.50±7.54 IU/L and for Sirohi bucks it was 86.16±3.50, 91.94±4.42 and 103.06±5.51 IU/L respectively.
There was no significant difference (P>0.05) between the two breeds of bucks with respect to GOT at pre freezing and post freezing (72 hours) stage in TYD dilutor. Significantly high GOT was noted in at post freezing (24 hours) stage in Osmanabadi bucks.

The results further indicated that in Osmanabadi bucks semen there was no significant difference (P>0.05) across different time interval and temperatures with respect to GOT in TYD dilutor. In Sirohi bucks semen there was significant difference between pre freezing and post freezing (72 hours) stage with respect to GOT leakage in TYD dilutor.

In both the breeds no significant difference in GOT was noted between post freezing 24 and 72 hours stage, which indicates sperm damage was not significantly increased post freezing in TYD dilutor.

From table 14 it is evident that GPT in TYD dilutors at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 19.49±1.08, 21.72±1.03 and 22.89±1.24 IU/L and for Sirohi bucks it was 20.38±0.94, 24.77±0.84 and 27.16±0.91 IU/L respectively.

There was a significant difference (P<0.05) between two breeds of bucks with respect to GPT at post freezing (24 and 72 hours) stages. Sirohi bucks semen showed higher GPT leakage at post freezing (24 and 72 hours) stages than Osmanabadi bucks.

There was significant difference (P<0.05) across different time interval and temperatures with respect to GPT in TYD dilutor.

The results further indicated that in Osmanabadi bucks semen there was significant difference between pre-freezing and 72 hours post-freezing GPT. Significantly high GPT leakage was noted at post freezing (72 hours) stage as compared to pre freezing in TYD dilutor.

In Sirohi bucks semen significant GPT leakage was noted between pre freezing and post freezing (24 and 72 hours) stage in TYD dilutor.

In both the breeds there was no significant leakage of GPT between 24 and 72 hours post-freezing; which indicates that GPT leakage was not significantly increased in both the breeds after freezing of semen.

On scanning the available literature, the references regarding trans-aminase post freezing in semen of Osmanabadi and Sirohi bucks in TYD dilutor at different
time interval and temperatures were not available. Therefore no comparison could be made.

4.5.8.6 GOT and GPT in TYE dilutor.

From table 10 it is evident that average GOT in TYE dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 112.22±8.01, 118.00±7.46 and 123.33±7.12 IU/L and for Sirohi bucks, it was 94.05±4.91, 106.22±5.43 and 121.22±7.25 IU/L respectively.

There was no significant difference (P>0.05) between post freezing (24 and 72 hours) stages with respect to GOT in the two breeds of bucks in TYE dilutor.

There was a significant difference (P<0.05) across different time interval and temperatures with respect to GOT in TYE dilutor.

The results further indicated that there was a no significant difference (P>0.05) across different time interval and temperatures with respect to GOT in TYE dilutor in Osmanabadi bucks.

In Sirohi bucks there was a significant difference between pre-freezing and 72 hours post-freezing stage with respect to GOT in TYE dilutor. Significantly higher GOT was noted at post freezing (72 hours) stage than pre-freezing (5°C) stage in TYE dilutor.

In both the breeds of bucks no significant difference in GOT was noted between 24 and 72 hours post-freezing, it indicates leakage GOT after 24 hours of semen freezing was stable which indicates that sperm damage was not increased after 24 hours of freezing.

From table 14 it is evident that the average GPT in TYE dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 22.72±1.11, 25.05±0.65 and 26.33±0.81 IU/L and for Sirohi bucks it was 24.05±1.22, 28.33±0.70 and 30.50±0.69 IU/L respectively.

There was a significant difference between two breeds of bucks at post freezing (24 and 72 hours) stages with respect to GPT in TYE dilutor. GPT was significantly higher at post freezing (24 and 72 hours) stages in Sirohi than Osmanabadi bucks.
There was a significant difference (P<0.05) across different time interval and temperatures with respect to GPT in TYE dilutor.

The results further indicated that in Osmanabadi bucks there was a significant difference between pre-freezing and 72 hours post-freezing GPT in TYE dilutor. Significantly, increased GPT noted was at 72 hours post-freezing than pre-freezing stage in Osmanabadi bucks in TYE dilutor.

In Sirohi bucks, there was a significant difference between pre-freezing and 24 hours post freezing as well as pre-freezing and 72 hours post-freezing GPT in TYE dilutor. In both the breeds, no significant difference in GPT was noted between 24 and 72 hours post-freezing, it indicates leakage of GOT after 24 hours of freezing was not increased significantly; means that sperm damage was not increased significantly after 24 hours of freezing.

On scanning the available literature, the references regarding post freezing GOT and GPT in TYE dilutor in semen of Osmanabadi and Sirohi bucks was not available, therefore no comparison could be made.
### 4.5.9 Alkaline phosphatase (AKP) and Acid phosphatase activity

The Alkaline phosphatase (AKP) and Acid phosphatase (ACP) activity at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks semen is given in table 15.

Table 11 Alkaline phosphatase (AKP) at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks

<table>
<thead>
<tr>
<th>Dilutor</th>
<th>AKP (IU/L)</th>
<th>C.V</th>
<th>F-value</th>
<th>ACP (IU/L)</th>
<th>C.V</th>
<th>F-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-freezing</strong></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>TYG(O)</td>
<td>1001.38±5.19\textsuperscript{hij}</td>
<td>Mean±S.E n=18</td>
<td>1.083</td>
<td>15.585*</td>
<td>Mean±S.E n=18</td>
<td>4.381</td>
</tr>
<tr>
<td>TYD(O)</td>
<td>1003.11±7.71\textsuperscript{ghi}</td>
<td>56.66±1.30\textsuperscript{fghi}</td>
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<td></td>
</tr>
<tr>
<td>TYE(O)</td>
<td>1008.76±6.02\textsuperscript{fgh}</td>
<td>55.72±1.44\textsuperscript{ghi}</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG(S)</td>
<td>987.00±3.04\textsuperscript{k}</td>
<td>57.55±1.47\textsuperscript{defghi}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD(S)</td>
<td>989.00±3.24\textsuperscript{k}</td>
<td>54.94±1.04\textsuperscript{hi}</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>TYE(S)</td>
<td>992.16±2.83\textsuperscript{jk}</td>
<td>56.05±1.79\textsuperscript{ghi}</td>
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<tr>
<td><strong>Post-freezing 24hr.</strong></td>
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<tr>
<td>TYG (O)</td>
<td>1026.33±3.91\textsuperscript{cde}</td>
<td>60.27±1.28\textsuperscript{bcd}</td>
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<tr>
<td>TYD (O)</td>
<td>1018.00±6.15\textsuperscript{def}</td>
<td>58.11±1.11\textsuperscript{cdefghi}</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>TYE (O)</td>
<td>1034.46±7.70\textsuperscript{abc}</td>
<td>60.66±0.98\textsuperscript{bcd}</td>
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<tr>
<td>TYG (S)</td>
<td>1003.88±4.28\textsuperscript{ghi}</td>
<td>62.16±0.82\textsuperscript{bcd}</td>
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<tr>
<td>TYD (S)</td>
<td>997.33±3.43\textsuperscript{hijk}</td>
<td>61.66±0.68\textsuperscript{bcd}</td>
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<td>TYE (S)</td>
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<td>63.22±0.90\textsuperscript{bcd}</td>
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<tr>
<td><strong>Post-freezing 72 hr</strong></td>
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</tr>
<tr>
<td>TYG (O)</td>
<td>1040.68±5.30\textsuperscript{ab}</td>
<td>62.50±1.73\textsuperscript{bdef}</td>
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<td>TYD (O)</td>
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<td>61.55±1.52\textsuperscript{c}</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE (O)</td>
<td>1046.73±4.99\textsuperscript{a}</td>
<td>64.11±1.32\textsuperscript{abcd}</td>
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<td></td>
</tr>
<tr>
<td>TYG (S)</td>
<td>1014.22±4.34\textsuperscript{efg}</td>
<td>66.55±0.84\textsuperscript{ab}</td>
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<tr>
<td>TYD (S)</td>
<td>1004.44±3.38\textsuperscript{ghi}</td>
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<td></td>
</tr>
<tr>
<td>TYE (S)</td>
<td>1020.11±4.76\textsuperscript{def}</td>
<td>69.55±0.72\textsuperscript{abc}</td>
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</tr>
</tbody>
</table>

O=Osmanabadi S=Sirohi ** significant at 5% level.
Means with common superscript do not differ significantly.
AKP C.D (0.05) = 12.579 ACP C.D (0.05) = 3.067
4.5.9.1 Alkaline phosphatase (AKP) and Acid phosphatase activity at pre-freezing (5°C) stage.

From table 11 it is evident that at pre-freezing stage (5°C) average AKP in TYG, TYD and TYE dilutors for Osmanabadi bucks was 1001.38±5.19, 1003.11±7.71 and 1008.76±6.02 IU/L and for Sirohi bucks it was 987.00±3.04, 989.00±3.24 and 992.16±2.83 IU/L respectively.

There was a significant difference (P<0.05) between the two breeds of bucks with respect AKP leakage at all freezing stages. Significantly, higher AKP leakage was noted in Osmanabadi than Sirohi bucks.

There was no significant difference (P>0.05) across different dilutors with respect to AKP at pre-freezing (5°C) stage. The leakage of AKP was not affected by different extenders.

Significantly, higher AKP leakage noted was in Osmanabadi than Sirohi bucks. Similar observation was reported by Roy et al. (1960) in cattle and buffalo bulls. They observed that phosphates levels were significantly higher in buffalo semen than the cattle semen.

From table 11 it is evident that at pre-freezing (5°C) stage average ACP in TYG, TYD and TYE dilutors for Osmanabadi bucks was 56.66±1.30, 55.72±1.44 and 57.55±1.47 IU/L and for Sirohi bucks it was 54.94±1.04, 56.05±1.79 and 58.27±0.50 IU/L respectively.

There was no significant difference (P>0.05) between the two breeds of bucks as well as across different dilutors with respect to ACP at pre-freezing (5°C) stage.

In present study, we had observed the higher levels of AKP than ACP at pre freeze stage in both breeds of bucks similar type of results were observed by Chauhan et al. (1994) in Jamnapari bucks.
4.5.9.2 Alkaline phosphatase (AKP) and Acid phosphatase activity at post-freezing (24 hours) stage

From table 11 it is evident that at post-freezing (24 hours) stage the average AKP in TYG, TYD and TYE dilutors for Osmanabadi bucks was 1026.33±3.19, 1018.00±6.15 and 1034.46±7.70 IU/L and for Sirohi bucks it was 1003.88±4.28, 997.33 ± 3.43 and 1002.55 ± 3.04 IU/L respectively.

There was a significant difference (P<0.05) between the two breeds of bucks with respective to AKP in all three dilutors at post-freezing (24 hours) stage. Significantly, higher leakage of AKP noted was in Osmanabadi than Sirohi bucks at post-freezing (24 hours) stage.

There was no significant difference across different dilutors with respect to AKP at post freezing (24 hours) stage.

From table 15 it is evident that at post-freezing (24 hours) stage the average ACP in TYG, TYD and TYE dilutors for Osmanabadi bucks was 60.27±1.28, 58.11±1.11 and 60.66±0.98 IU/L and for Sirohi bucks it was 62.16±0.82, 61.66±0.68 and 63.22±0.90 IU/l respectively.

There was a significant difference (P<0.05) between the two breeds of bucks with respect to ACP at post-freezing (24 hours) stage in TYD dilutor. Significantly higher ACP noted was in Sirohi than Osmanabadi bucks.

There was no significant difference (P>0.05) across different dilutors with respect to ACP at post-freezing (24 hours) stage. Dilutors did not affect ACP at post-freezing (24 hours) stage.

Literature pertaining to buck semen preservation especially for AKP and ACP post-freezing leakage was scanty; hence comparison was made with other species.

Dhami et al. (1990) compared enzyme leakage in Tris fructose egg yolk – glycerol diluter. The average AKP release Surti buffalo bull seminal plasma was 69.26±6.12 and 71.30±10.39 KAU/100ml respectively at post thaw. The average ACP release was 48.37±3.49 and 59.51±6.86 KAU/100ml respectively at post thaw stage. They noted the higher activity of AKP than ACP, similar trend observed was in both the breeds of bucks in present study.
Singh *et al.* (1992) also observed the higher leakage of AKP than ACP on freezing buffalo bull semen, which was in agreement with present study.

Chauhan *et al.* (1994) opined that AKP and ACP leakage in frozen thawed semen had positive correlation with acrosomal damage in Jamanpari bucks. Similarly in present study also higher AKP leakage was noted in Osmanabadi bucks where higher acrosomal damage was noted.

Similar to present study Sirat *et al.* (1996) observed a Leakage of AKP and ACP enzyme found was to increase from the pre-freeze to the post-freeze stage.

### 4.5.9.3 Alkaline phosphatase (AKP) and Acid phosphatase activity at post-freezing (72 hours) stage.

From table 11 it is evident that at post-freezing (72 hours) stage average AKP in TYG, TYD and TYE dilutors for Osmanabadi bucks was 1040.68±5.30, 1028.66±6.24, 1046.73±4.99 and for Sirohi bucks it was 1014.22±4.34, 1004.44±3.38, 1020.11±4.76 IU/L respectively.

There was a significant difference (P<0.05) between the two breeds of bucks with respect to AKP at post freezing (72 hours) stage. Significantly high ACP was noted in Osmanabadi bucks in TYE dilutor.

There was significant difference (P<0.05) across different dilutors with respect to AKP at post freezing (72 hours) stage.

The results further indicated that in both the breeds there was a significant difference between TYD and TYE dilutors with respect to AKP at post freezing (72 hours) stage. AKP leakage was more in TYE than TYD dilutor. No significant AKP leakage noted was in TYG and TYD as well as TYG and TYE dilutors. This indicates that AKP leakage was less in TYD and TYG as compared to TYE dilutor.

From table 11 it is evident that at post-freezing (72 hours) stage average ACP in TYG, TYD and TYE dilutors for Osmanabadi bucks was 62.50±1.73, 61.55±1.52 and 64.11±1.32 and Sirohi bucks it was 66.55±0.84, 64.50±1.08 and 69.55±0.72 IU/L respectively.
There was a no significant difference (P>0.05) between the two breeds of bucks with respect to ACP at post-freezing (72 hours) stage.

There was no significant difference in both the breeds across different dilutors with respect to ACP at post-freezing (72 hours) stage.

Murdoch and white (1968) reported that enzymes such as ACP and AKP were leached out of ram and bull spermatozoa by deep-freezing. Similar observation was also reported for both the breeds of bucks in present study.

4.5.9.4 Alkaline phosphatase (AKP) and Acid phosphatase activity in TYG dilutor

From table 11 it is evident that the average Alkaline phosphates (AKP) in TYG dilutor at pre-freezing (5°C), post freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 1001.38±5.19, 1026.33±3.91 and 1040.68±5.30 IU/L and for Sirohi bucks it was 987.00±3.04, 1003.88±4.28, 1014.22±4.34 IU/L respectively.

There was significant difference (P<0.05) between the two breeds of bucks at pre-freezing and post freezing (24 and 72 hours) stages. Significantly higher AKP noted was in Osmanabadi than Sirohi bucks.

There was significant difference (P<0.05) at different time interval and temperature with respect to AKP in TYG dilutor.

The results further indicated that in Osmanabadi bucks there was a significant difference across pre-freezing and 24 hours post-freezing, pre-freezing and 72 hours post freezing as well as 24 and 72 hours post-freezing stages with respect to AKP in TYG dilutor. This indicates that significant AKP leakage continued after semen freezing. Further investigation is necessary to know up to what time the AKP leakage continues at post-freezing stage in Osmanabadi bucks.

In Sirohi bucks, there was a significant difference across pre-freezing and 24 hours post-freezing as well as pre-freezing and post freezing (72 hours) stages but there was no significant difference between 24 hours post-freezing and 72 hours post-freezing stage with respect to AKP in TYG dilutor.
From table 11 it is evident that the average Acid phosphatase (ACP) in TYG dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 56.66±1.30, 60.27±1.28 and 62.50±1.73 IU/L and for Sirohi bucks it was 54.94±1.04, 62.16±0.82, and 66.55±0.84 IU/L respectively.

There was no significant difference (P<0.05) between Osmanabadi and Sirohi bucks in with respect to ACP in TYG dilutor.

There was a significant difference (P<0.05) between time interval and temperature with respect to ACP in TYG dilutor.

The results further indicated that in Osmanabadi bucks semen there was no significant difference across different time interval and temperature with respect to ACP in TYG dilutor.

In Sirohi bucks semen there was a significant difference across pre-freezing and post freezing (24 hours) as well as pre freezing and post-freezing (72 hours) stage with respect to ACP in TYG dilutor. But there was no significant difference across post freezing 24 and 72 hours with respect to ACP in TYG dilutor.

On scanning available literature, references regarding post-freezing leakage of AKP and ACP especially in TYG dilutor were not found so comparison could not be made.

4.5.9.5 Alkaline phosphatase (AKP) and Acid phosphatase activity in TYD dilutor

From table 11 it is evident that the average Alkaline phosphatase (AKP) in TYD dilutor at pre-freezing (5°C), post-freezing (24 hours) and post freezing (72 hours) stages for Osmanabadi bucks was 1003.66±7.71, 1018.00±6.15 and 1028.72±6.24 IU/L and for Sirohi bucks it was 989.00±3.24, 997.33±3.43 and 1004.44±3.38 IU/L respectively.

There was a significant difference (P<0.05) between two breeds of bucks semen with respect to AKP at pre freezing and post freezing stages. Higher AKP leakage noted was in Osmanabadi than Sirohi bucks in TYD dilutor.

There was significant difference noted (P<0.05) across different time interval and temperatures with respect to AKP in TYD dilutor.

The results further indicated that in Osmanabadi bucks semen there was a significant difference between pre-freezing and post freezing (24 and 72 hours)
stages, AKP significantly increased at post freezing (24 and 72 hours) stages than pre-freezing (5⁰C) stage.

In Sirohi bucks semen there was a significant difference between pre-freezing and post freezing (72 hours) stage AKP in TYD dilutor. AKP significantly increased at post freezing (72 hours) stage than pre-freezing (5⁰C) stage.

No significant AKP leakage observed between 24 and 72 hours post-freezing in TYD dilutor in both the breeds semen, which indicates that AKP leakage was not significantly increased post-freezing in TYD dilutor.

From table 11 it is evident that ACP in TYD dilutor at pre-freezing (5⁰C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 55.72±1.44, 58.11±1.11 and 61.55±1.52 IU/L and for Sirohi bucks it was 56.05±1.79, 61.66±0.68 and 64.50±1.08 IU/L respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to ACP at pre freezing and post freezing (24 and 72 hours) stages in TYD dilutor.

There was a significant difference across different time interval and temperatures with respect to ACP in TYD dilutor.

The results further indicated that in both the breeds of buck semen there was a significant difference between pre-freezing and 72 hours post freezing ACP in TYD dilutor. No significant difference noted between post freezing 24 and 72 hours ACP in both breeds semen in TYD dilutor, which indicates that in TYD dilutor ACP leakage was not significant after freezing.

On scanning the available literature, the references regarding AKP and ACP post freezing in semen of Osmanabadi and Sirohi bucks in TYD dilutor at different time interval and temperatures were not available. Therefore no comparison could be made.

4.5.9.6 Alkaline phosphatase (AKP) and Acid phosphatase activity in TYE dilutor

From table 11 it is evident that the average Alkaline phosphatase (AKP) in TYE dilutors at pre-freezing (5⁰C), post-freezing (24 hours) and post-freezing (72
hours) stages for Osmanabadi bucks was 1008.76±6.02, 1034.46±7.70, 1046.73±4.99 IU/L and for Sirohi bucks it was 992.16±2.83, 1002.55±3.04 and 1020.11±4.76 IU/L respectively.

There was a significant difference (P<0.05) between the two breeds of bucks at different time interval and temperatures. AKP was significantly higher in Osmanabadi than Sirohi bucks at pre-freezing and post freezing (24 and 72 hours) stages in TYE dilutor.

There was a significant difference (P<0.05) between different time interval and temperatures with respect to AKP in TYE dilutor.

The results further indicated that in Osmanabadi bucks semen there was significant difference across pre-freezing and post freezing (24 hours) as well as pre-freezing and post freezing (72 hours) stages with respect to AKP in TYE dilutor. Significantly, higher AKP noted was at post freezing 24 and 72 hours than pre-freezing stage. No significant difference was noted between post freezing (24 hours) and post freezing (72 hours) stage, indicates that AKP was not significantly increased after 24 hours of freezing in Osmanabadi bucks indicating no significant increase in sperm damage.

In Sirohi bucks, there was significant difference between pre-freezing and post freezing (72 hours) as well as post freezing (24 hours) and post freezing (72 hours) stage with respect AKP in TYE dilutor. As there was significant increase in AKP post freezing (72 hours) than post freezing (24 hours) indicates that sperm damage was significantly increasing in Sirohi bucks post freezing (72 hours) stage in TYE dilutor.

From Table 11 it is evident that the average Acid phosphatase (ACP) in TYE dilutors at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stage for Osmanabadi bucks was 57.55±1.47, 60.66±0.98, 64.11±1.32 IU/L and for Sirohi bucks it was 58.27±0.50, 63.22±0.90 and 69.55±0.72 IU/L respectively.

There was no significant difference (P>0.05) between two breeds of bucks with respect to ACP in TYE dilutor.

There was no significant difference (P>0.05) across different time interval and temperatures with respect to ACP in TYE dilutor.
On scanning the available literature, the references regarding post freezing AKP and ACP in TYE dilutor in semen of Osmanabadi and Sirohi bucks was not available, therefore no comparison could be made.
4.5.10 Lactate dehydrogenase (LDH) activity

The Lactate dehydrogenase (LDH) at different freezing stages in different dilutors of Osmanabadi and Sirohi bucks semen is given in table 12

Table 12 Lactate dehydrogenase (LDH) at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks semen.

<table>
<thead>
<tr>
<th>Dilutor</th>
<th>LDH (IU/L)</th>
<th>C.V</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-freezing</td>
<td>Mean±S.E, n=18</td>
<td>5.644</td>
<td>1.917**</td>
</tr>
<tr>
<td>TYG(O)</td>
<td>308.26±5.02\textsuperscript{bcde}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD(O)</td>
<td>309.25±4.34\textsuperscript{bcde}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE(O)</td>
<td>315.56±5.51\textsuperscript{abde}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG(S)</td>
<td>297.27±8.47\textsuperscript{e}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (S)</td>
<td>298.05±8.92\textsuperscript{e}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE(S)</td>
<td>302.61±8.42\textsuperscript{de}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-freezing 24 hr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG (O)</td>
<td>320.83±6.03\textsuperscript{abcd}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (O)</td>
<td>319.55±4.49\textsuperscript{abcd}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE (O)</td>
<td>321.06±5.76\textsuperscript{abcd}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG (S)</td>
<td>306.66±8.24\textsuperscript{cde}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (S)</td>
<td>310.49±9.75\textsuperscript{bde}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE (S)</td>
<td>308.72±9.71\textsuperscript{bcde}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-freezing 72 hr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG (O)</td>
<td>325.88±5.31\textsuperscript{abc}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (O)</td>
<td>324.18±4.83\textsuperscript{abc}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE (O)</td>
<td>331.50±5.62\textsuperscript{a}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYG (S)</td>
<td>320.55±8.33\textsuperscript{abcd}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYD (S)</td>
<td>316.83±11.54\textsuperscript{abcd}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYE (S)</td>
<td>327.27±11.49\textsuperscript{ab}</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

O=Osmanabadi  S= Sirohi * significant at 5% level, C.D (0.05) = 20.402
Means with common superscript do not differ significantly.
4.5.10.1 Lactate dehydrogenase (LDH) at pre-freezing (5°C) stage

From table 12 it is evident that at pre-freezing (5°C) stage the average LDH in TYG, TYD and TYE dilutors for Osmanabadi bucks was 308.26±5.02, 309.25±4.34 and 315.56±5.51 IU/L and for Sirohi bucks it was 297.27±8.47, 298.05±8.92 and 302.61±8.42 IU/L respectively.

There was no significance difference (P>0.05) between the two breeds of bucks with respect to LDH at pre-freezing (5°C) stage.

There was no significance difference (P>0.05) across different dilutors with respect to LDH at pre-freezing (5°C) stage. This indicates that LDH was not affected by dilutors in both the breeds.

Dhami (1986) in semen of Surti buffalo bulls (114.61±5.77 IU/L), Dhami et al. (1990) in Surti buffalo bulls (120.85±11.92 and 105.75±14.83 IU/L) reported lower levels of LDH in pre freeze seminal plasma than present observations

Singh et al. (1991) recorded LDH activity of seminal plasma in Murrah bulls in TYG at equilibration (81.40±1.82 n mol pyruvate reduce/min/ml). Zamiri and Heidari (2006) in Rayini male goats of Kerman province in Iran recorded seasonal variation in LDH activity. Their data indicated that lowest level of lactate dehydrogenase in the seminal fluid was recorded in early September (2.2 U/mL) and the highest level in November (2.5 U/mL). But due to difference in method of expression of units the actual observations could not be compared.

4.5.10.2 Lactate dehydrogenase (LDH) at post-freezing (24 hours) stage

From table 12 it is evident that at post-freezing stage (24 hours) the average LDH in TYG, TYD and TYE dilutors for Osmanabadi bucks was 320.83±6.03, 319.55±4.49 and 321.06±5.76 IU/L and for Sirohi bucks it was 306.66±8.24, 310.49±9.75 and 308.7±9.71 IU/L respectively.
There was no significance difference (P>0.05) between the two breeds of bucks as well as across different dilutors with respect to LDH at post-freezing (24 hours) stage.

Dhami (1986) in Surti buffalo bulls, Dhami and Kodagali (1990) in Surati buffalo bulls, Singh et al. (1990) in Murrah bulls observed that LDH in seminal plasma was increased after freezing in surti buffalo bulls, this trend was in agreement with present observations for both breed of bucks.

Sirat et al. (1996) reported that in bucks activity of lactic dehydrogenase was significantly higher in extenders containing DMSO than lactose. Leakage of this enzyme was found to increase from the pre-freeze to the post-freeze stage. In present study also similar trend was noted, but the LDH was not higher in TYD dilutor than other two dilutors. This could be attributed to the percentage of DMSO and buffer used for semen preservation.

Zamiri and Heidari (2006) in Rayini male goats recorded low LDH activity in early September (2.2 U/ml) and the highest level in November (2.5 U/ml).

4.5.10.3 Lactate dehydrogenase (LDH) at post-freezing (72 hours) stage

From table 12 it is evident that at post-freezing stage (72 hours) the average LDH in TYG, TYD and TYE dilutors for Osmanabadi bucks was 325.88±5.31, 324.2±4.83 and 331.50±5.62 IU/L and for Sirohi bucks it was 320.55±8.33, 316.83±11.54 and 327.27±11.49 IU/L respectively.

There was no significant difference between the two breeds of bucks as well as across different dilutors with respect to LDH at post freezing (72 hours) stage.

Murdoch and White (1968) reported that on deep freezing of semen there was leakage of LDH in ram and bull, which was in agreement with present observations.
4.5.10.4 Lactate dehydrogenase (LDH) in TYG dilutor

From table 12 it is evident that the average LDH in TYG dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stage for Osmanabadi bucks was 56.33±1.30, 60.33±1.28 and 62.50±1.73 IU/L and for Sirohi bucks it was 54.94±1.04, 62.17±0.82 and 66.56±0.84 IU/L respectively.

There was no significant difference between the two breeds of bucks as well as there was no significance difference across different time interval and temperatures respect to LDH in TYG dilutor.

On scanning available literature references regarding post-freezing leakage of LDH specially in TYG diluter were not found so comparison could not be made.

In TYG dilutor, the quality of semen at all three stage of freezing of was good. The seminal attributes reduced gradually from pre-freeze to post freeze stage but they were in normal prescribed limit.

4.5.10.5 Lactate dehydrogenase (LDH) in TYD dilutor

From table 12 it is evident that the average LDH in TYD dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 309.25±4.34, 318.67±4.49 and 324.19±4.83IU/L and for Sirohi bucks it was 298.05±8.92, 310.50±9.75, 316.83±11.54 IU/L respectively.

There was no significant difference (P>0.05) between the two breeds of bucks as well as across different time interval and temperatures with respect to LDH in TYD diluter. LDH was not affected pre and post-freezing stages in TYD dilutor in semen of Osmanabadi and Sirohi bucks.

On scanning available literature references regarding post-freezing leakage of LDH especially in TYD diluter were not found so comparison could not be made.
4.5.10.6 Lactate dehydrogenase (LDH) in TYE dilutor

From table 12 it is evident that average LDH in TYE dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 315.55±5.51, 321.17±5.76 and 331.50±5.62 IU/L and for Sirohi bucks it was 302.61±8.42, 308.72±9.71, 327.28±11.49 IU/L respectively.

There was no significance difference (P>0.05) between the two breeds of bucks as well as there was no significance difference (P>0.05) across different time interval and temperatures with respect to LDH in TYE dilutors except in Sirohi bucks there was significant leakage of LDH from pre freezing to post freezing (72 hours) stage.

On scanning the available literature, the references regarding post freezing LDH in TYE dilutor in semen of Osmanabadi and Sirohi bucks was not available, therefore no comparison could be made.
4.6. Study of conception rate of frozen semen

4.6.1 Conception rate of Osmanabadi buck frozen semen

From table 13 it is evident that the conception rate of Osmanabadi bucks semen frozen in straws in TYG, TYD and TYE dilutor in oestrous synchronized does was 33.33, 38.88 and 19.44 percent respectively. The overall conception rate for Osmanabadi bucks frozen semen was 30.55 percent.

Table 13 Conception rate of Osmanabadi buck frozen semen in different dilutors

<table>
<thead>
<tr>
<th>Buck No</th>
<th>TYG (Pregnant goats)</th>
<th>TYD (Pregnant goats)</th>
<th>TYE (Pregnant goats)</th>
<th>Buck wise pregnant goats</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>02</td>
<td>03</td>
<td>02</td>
<td>07</td>
</tr>
<tr>
<td>2</td>
<td>02</td>
<td>02</td>
<td>01</td>
<td>05</td>
</tr>
<tr>
<td>4</td>
<td>03</td>
<td>02</td>
<td>01</td>
<td>06</td>
</tr>
<tr>
<td>5</td>
<td>02</td>
<td>02</td>
<td>01</td>
<td>05</td>
</tr>
<tr>
<td>6</td>
<td>02</td>
<td>03</td>
<td>01</td>
<td>06</td>
</tr>
<tr>
<td>9</td>
<td>01</td>
<td>02</td>
<td>01</td>
<td>04</td>
</tr>
<tr>
<td>Total goat pregnant</td>
<td>12 (n=36)</td>
<td>14 (n=36)</td>
<td>07 (n=36)</td>
<td>33 (n=108)</td>
</tr>
<tr>
<td>Conception Rate (%)</td>
<td>33.33</td>
<td>38.88</td>
<td>19.44</td>
<td>30.55</td>
</tr>
</tbody>
</table>

n= number of oestrous synchronized does inseminated.

Higher conception rate was observed for semen frozen in TYD dilutor as compared with TYE and TYG dilutor. Higher conception rate was also observed for TYG as compared to TYE dilutor. It indicates that DMSO was best for freezing Osmanabadi bucks semen in straws compared to glycerol and ethylene glycol.
4.6.2 Conception rate of Sirohi buck frozen semen

From table 14 it is evident that the conception rate of Sirohi bucks semen frozen in straws in TYG, TYD and TYE dilutor in oestrous synchronized does was 30.55, 36.11 and 27.77 percent respectively. The overall conception rate for Sirohi buck frozen semen was 31.48 percent.

**Table 14 Conception rate of Sirohi buck frozen semen in different dilutors**

<table>
<thead>
<tr>
<th>Buck No</th>
<th>TYG (Pregnant goats)</th>
<th>TYD (Pregnant goats)</th>
<th>TYE (Pregnant goats)</th>
<th>Buck wise Pregnant goats</th>
</tr>
</thead>
<tbody>
<tr>
<td>13049</td>
<td>01</td>
<td>02</td>
<td>01</td>
<td>04</td>
</tr>
<tr>
<td>13087</td>
<td>02</td>
<td>02</td>
<td>02</td>
<td>06</td>
</tr>
<tr>
<td>13042</td>
<td>01</td>
<td>02</td>
<td>01</td>
<td>04</td>
</tr>
<tr>
<td>13086</td>
<td>02</td>
<td>03</td>
<td>01</td>
<td>06</td>
</tr>
<tr>
<td>13062</td>
<td>03</td>
<td>02</td>
<td>02</td>
<td>07</td>
</tr>
<tr>
<td>13097</td>
<td>02</td>
<td>02</td>
<td>03</td>
<td>07</td>
</tr>
<tr>
<td>Total goat pregnant</td>
<td>11 (n=36)</td>
<td>13 (n=36)</td>
<td>10 (n=36)</td>
<td>34 (n=108)</td>
</tr>
</tbody>
</table>

Conception Rate (%) 30.55 36.11 27.77 31.48

n= number of oestrous synchronized does inseminated.

Higher conception rate was observed for semen frozen in TYD dilutor as compared with TYE and TYG dilutor. Higher conception rate was also observed for TYG as compared to TYE dilutor. It indicates that DMSO was best for freezing Sirohi bucks semen compared to glycerol and ethylene glycol.
Figure 13 Osmanabadi X Sirohi kids born through AI

Figure 14 Osmanabadi X Boer kids born through AI

Figure 15 Osmanabadi X Damascus kid born through AI
4.6.3 Overall conception rate of Osmanabadi and Sirohi buck frozen semen in different dilutors

From table 15 it is evident that average conception rate of semen frozen in TYG, TYD and TYE dilutors for Osmanabadi bucks was 33.33, 36.11 and 19.44 and for Sirohi bucks it was 30.55, 38.88 and 27.77 percent respectively.

<table>
<thead>
<tr>
<th>Breed/Dilutor</th>
<th>TYG (n=72)</th>
<th>TYD (n=72)</th>
<th>TYE (n=72)</th>
<th>Total Conception rate (n=216)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmanabadi</td>
<td>33.33</td>
<td>38.88</td>
<td>19.44</td>
<td>30.55</td>
</tr>
<tr>
<td>Sirohi</td>
<td>30.55</td>
<td>36.11</td>
<td>27.77</td>
<td>31.48</td>
</tr>
<tr>
<td>Conception Rate</td>
<td>31.94</td>
<td>37.49</td>
<td>23.60</td>
<td>31.01</td>
</tr>
</tbody>
</table>

n= number of oestrous synchronized does inseminated.

The overall conception rate of semen frozen in TYG, TYD and TYE dilutors was 31.94, 37.49 and 23.60 % respectively. Higher conception rate was observed for TYD dilutor than TYG and TYE dilutors. TYG dilutor also showed higher conception rate than TYE dilutor. It indicates that TYD was the best dilutor followed by TYG for freezing Osmanabadi and Sirohi buck semen. This also indicates that fertility of spermatozoa was better preserved when DMSO was used as cryoprotectant than glycerol or ethylene glycol.

The conception rates using frozen buck semen in different extenders in the range of 35 to 40 percent observed in different breeds of goats by Corteel et al. (1974), Ritar et al. (1990), Ritar and Ball (1993), Gacitua and Arav (2005), Menchaca and Rubianes (2007), Dogan et al. (2008) and Batista et al. (2009) were in agreement with present findings.

However, the conception rate using frozen buck semen in different extenders above 40 percent was observed in different breeds of goats by Corteel et al. (1980), Lawrenz (1986), Chauhan and Anand(1990), Bakshi (1991), Sinha et al. (1995), Karatzas et al. (1997), Greyling and Nest (2000), Salvador et al. (2005), Khalifa and
Saidy (2006), Salvador et al. (2006), Mara et al. (2007), Leboeuf et al. (2008), and El-Kon et al. (2010) was higher than present findings. The difference in observations may be due to variation in breeds, dilutors used, fertility of female etc. According to Salvador et al. (2005) the factors such as depth of semen deposited, inseminator skill, farm, sire, expression of oestrous etc also affect the conception rate in does.

4.7 Evaluation of Boer and Damascus bucks pelleted frozen semen received from research organization

4.7.1 Evaluation of semen quality of pellets of Boer and Damascus breed of bucks

From table 16 it is evident that in Boer and Damascus bucks frozen semen average microscopic semen attributes such as post thaw sperm motility percentage was 65.00±1.82 and 65.00±2.24, live sperm percentage was 74.67±1.14 and 77.50±2.25, abnormal sperm percentage was 19.00±0.97 and 20.17±0.83 respectively.

From table 16 it is evident that in Boer and Damascus bucks frozen semen average sperm function test attributes such as hypo osmotic swollen sperm percentage was 64.33±1.48 and 64.67±1.52, acrosome intact sperm percentage was 85.67±1.71 and 85.33±2.26, DNA damaged sperm percentage by acridine orange test was 8.83±0.60 and 11.50±0.99 and DNA damaged sperm percentage by Comet assay was 0.83±0.40 and 1.00±0.37 respectively.

From table 16 it is evident that in Boer and Damascus bucks frozen semen average GOT was 80.33±0.95 and 85.67±0.92 IU/L, GPT was 19.33±1.16 and 18.33±0.95 IU/L, AKP was 889.17±8.44 and 955.17±14.22 IU/L, ACP was 46.00±2.00 and 45.50±1.26 IU/L and LDH was 301.17±4.18 and 313.00±4.76 IU/L respectively.
Table 16 Quality of Boer and Damascus bucks pelleted frozen semen

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Boer Mean ± S.E, n=06</th>
<th>Damascus Mean ± S.E, n=06</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Motility percentage</td>
<td>65.00±1.82</td>
<td>65.00±2.24</td>
</tr>
<tr>
<td>Live sperm percentage</td>
<td>74.67±1.14</td>
<td>77.50±2.25</td>
</tr>
<tr>
<td>Abnormal Sperm percentage</td>
<td>19.00±0.97</td>
<td>20.17±0.83</td>
</tr>
<tr>
<td>HOST percentage</td>
<td>68.17±1.48</td>
<td>60.17±1.52</td>
</tr>
<tr>
<td>Acrosome intactness percentage</td>
<td>85.67±1.71</td>
<td>85.33±2.26</td>
</tr>
<tr>
<td>DNA damage percentage by AO</td>
<td>8.83±0.60</td>
<td>11.50±0.99</td>
</tr>
<tr>
<td>DNA damage percentage by comet assay</td>
<td>0.83±0.40</td>
<td>1.00±0.37</td>
</tr>
<tr>
<td>GOT (IU/L)</td>
<td>80.33±0.92</td>
<td>85.17±0.95</td>
</tr>
<tr>
<td>GPT (IU/L)</td>
<td>19.33±1.16</td>
<td>18.33±0.95</td>
</tr>
<tr>
<td>AKP (IU/L)</td>
<td>889.17±8.44</td>
<td>955.17±14.22</td>
</tr>
<tr>
<td>ACP (IU/L)</td>
<td>46.00±2.00</td>
<td>45.50±1.26</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>301.17±4.18</td>
<td>313.00±4.</td>
</tr>
</tbody>
</table>

The values reported in table 16 regarding different parameters of Boer and Damascus bucks frozen semen indicates that the quality of frozen semen from these two breed of bucks was good.

Khalifa and Saidy (2006) recorded 53.89 ± 4.31 percent post thaw sperm motility in semen of Damascus bucks frozen by pellet method. This observation was lower than present findings. They had also observed that post thaw sperm acrosomal damage was 54.33±2.07 which was higher than present observations. This may be due to difference in dilutors used, staining technique etc.
4.7.2 Conception rate of Boer and Damascus bucks pelleted frozen semen

From table 17 it is evident that the average percentage of conception rate of Boer and Damascus bucks pelleted frozen semen was 25 and 16.66 percent respectively.

Table 17 Conception rate of Boer and Damascus buck pelleted frozen semen

<table>
<thead>
<tr>
<th></th>
<th>Boer</th>
<th>Damascus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total goat inseminated</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Pregnant goat</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Conception rate</td>
<td>25%</td>
<td>16.66%</td>
</tr>
</tbody>
</table>

The Boer buck frozen semen had showed higher conception rate as compared to Damascus buck frozen semen.

Khalifa and Saidy (2006) obtained 55.26% kidding rates in natural and 53.85% in synchronized oestrus in does using pelleted frozen semen for artificial insemination in does. This was higher than present study. This may be attributed to multiple factor like breed of goats, season, time of AI, fertility status of does, oestrous exhibition, plane of nutrition etc.
5. SUMMARY AND CONCLUSIONS

The present research work was carried out at the Instructional Livestock Farm Complex, National Institute for Research in Reproductive Health (NIRRH), Parel, Mumbai-12, Department of Animal Reproduction Gynecology and Obstetrics, Bombay Veterinary College and Frozen Semen laboratory, Kokan Development Corporation Ltd, Aarey Colony, Unit No.16, Goregaon (East), Mumbai-400 065. Total 12 bucks (six Osmanabadi and six Sirohi) belonging to Instructional Livestock Farm Complex were used for the research work. Semen evaluation was carried at laboratory of Livestock Farm and NIRRH. Semen freezing and preservation was done at frozen semen laboratory, Kokan Development Corporation Ltd. Artificial Insemination trials were performed on goats of Instructional Livestock Farm Complex and Punya Shlok Ahilyadevi Sheep and Goat Farm, Tirth (Budruk), Taluka Tuljapur, Dist Solapur, Maharashtra state.

Semen evaluation

Semen samples of 12 bucks (6 Osmanabadi and 6 Sirohi) were evaluated for various macroscopic and microscopic tests. Three samples were collected in all bucks at an interval of 7-15 days period. The repeat sample was treated as a new sample and the observations for seminal attributes were pooled for statistical analysis. Semen was collected in clean graduated glass collection cups by artificial vagina method. After collection volume, colour, consistency, density were evaluated by visual observation. pH was noted by using pH paper. Semen was kept in a water bath at 37°C, till further evaluation was carried out.
5.1 Macroscopic evaluation of semen of Osmanabadi and Sirohi bucks.

5.1.1 Ejaculate volume

The average ejaculate volume for Osmanabadi and Sirohi buck was 1.7±0.11 ml and 1.8±0.07 ml respectively. There was no significant difference observed in the ejaculate volume between two breeds.

5.1.2 Colour, Consistency and Density of neat semen.

The colour of semen varied from yellowish to yellowish white in Osmanabadi bucks, whereas it varied from yellowish white to creamy white in Sirohi bucks.

5.1.3 Hydrogen ion concentration (pH) of semen

The average pH of semen of Osmanabadi and Sirohi bucks was 6.70±0.03 and 6.65±0.02 respectively. There was no significant difference between the two breeds with respect to pH of neat semen.

5.1.4 Resazurin test

The average total time required for changing colour from blue to white in resazurin test of neat semen of Osmanabadi and Sirohi buck semen was 15.96±0.66 and 19.3±0.13 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to resazurin test.
5.2. Microscopic evaluation of semen of Osmanabadi and Sirohi bucks

5.2.1 Mass activity

The average mass activity of Osmanabadi and Sirohi buck semen was in the grade of + 4.

There was no significant difference (P>0.05) between mass activities of both the breeds of bucks.

5.2.2 Total sperm concentration

The total sperm concentration of Osmanabadi and Sirohi buck neat semen was 2616.80 ± 39.12 and 2364.70± 75.50 million/ml respectively.

There was a significant difference (P<0.05) between the two breeds with respect to total sperm concentration. The sperm concentration was higher in case of Osmanabadi than Sirohi bucks.

5.2.3. Initial sperm motility percent in neat semen.

The average initial sperm motility percentage of Osmanabadi and Sirohi bucks semen was 86.66 ±1.72 and 86.13 ± 1.33 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to initial motility.

5.2.4 Live sperm percentage in neat semen.

The live sperm percentage of Osmanabadi and Sirohi bucks semen was 86.05 ± 0.41 and 84.05 ± 0.79 respectively.
There was a significant difference (P<0.05) between the two breeds of bucks with respect to live sperm percentage. Significantly higher percentages of live spermatozoa were noted in neat semen of Osmanabadi than Sirohi bucks.

5.2.5 Abnormal sperm percentage in neat semen.

The abnormal sperm percentage in Osmanabadi and Sirohi bucks semen was 13.28 ± 1.09 and 11.78 ± 0.90 respectively.

There was no significant difference (P>0.05) between the two breeds with respect to abnormal sperm percentage.

5.3 Evaluation of neat semen of Osmanabadi and Sirohi bucks using sperm function tests

5.3.1 Plasma membrane integrity (Hypo Osmotic Swelling Test- HOST)

It was evident that 83.13 ± 1.18 and 78.33 ± 1.78 percentage of spermatozoa had shown swelling under HOS test in Osmanabadi and Sirohi bucks neat semen respectively.

There was a significant difference (P<0.05) between the two breeds of bucks with respect to HOS sperm percent. The Osmanabadi buck semen showed significantly higher plasma membrane integrity in neat semen as compared to Sirohi bucks.

5.3.2 Acrosome integrity (Giemsa stain)

It was evident that the average acrosome intact sperm percentage of Osmanabadi and Sirohi bucks neat semen was 91.94 ± 0.75 and 90.94 ± 1.24 respectively.
There was no significant difference (P>0.05) between the two breeds of bucks with respect to Acrosome Intactness in neat semen.

5.3.3 DNA damage

5.3.3.1 DNA damaged sperm percentage (Acridine orange test)

No DNA damaged sperm were detected in Osmanabadi and Sirohi bucks neat semen using Acridine Orange test.

5.3.3.2 DNA damaged sperm percentage (Comet assay)

No DNA damaged sperm were detected in Osmanabadi and Sirohi bucks neat semen using comet assay test.

5.4 Enzyme in seminal plasma of Osmanabadi and Sirohi bucks.

5.4.1 Transaminase (GOT and GPT)

The average Glutamic- Oxaloacetic Transaminase (GOT) in seminal plasma of Osmanabadi and Sirohi buck semen was 82.55±6.95 and 73.61±4.37IU/l respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to GOT in neat semen.

The average Glutamic- Pyruvate Transaminase (GPT) in seminal plasma of Osmanabadi and Sirohi buck was 16.94 ±0.84 and 16.06±0.81 IU/l respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to GPT in neat semen.
5.4.2 Alkaline phosphatase (AKP) and Acid Phosphatase (ACP)

The average of AKP in seminal plasma of Osmanabadi and Sirohi buck was 970.9±5.39 and 974.1±4.24 IU/l respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to AKP.

The average of ACP in seminal plasma of Osmanabadi and Sirohi bucks was 48.95 and 47.38 IU/l respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to ACP.

5.4.3 Lactate dehydrogenase (LDH)

The average LDH in seminal plasma of Osmanabadi and Sirohi bucks was 293.4±9.28 and 281.6±6.43 IU/l respectively.

There was no significance difference (P>0.05) between the two breeds of bucks with respect to LDH in neat semen.
5.5 Evaluation of semen quality of Osmanabadi and Sirohi bucks at different freezing stages in different dilutors.

5.5.1 Sperm Motility

The sperm motility percentage at different freezing stages in different dilutors was recorded as below.

5.5.1.1 Sperm motility percentage at pre-freezing (5°C) stage

The average sperm motility percentage of semen at pre-freezing (5°C) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 82.22±1.73, 83.33±2.11 and 77.78±2.68 and for Sirohi bucks it was 71.66±1.43, 74.45±1.65 and 66.65±1.72 respectively.

There was a significant difference (P<0.05) between the two breeds of bucks with respect to sperm motility percentage at pre-freezing stage. The sperm motility was significantly higher in Osmanabadi than Sirohi bucks.

There was a significant difference (P<0.05) across different dilutors with respect to sperm motility percentage at pre-freezing stage.

The results further indicated that there was a significant difference between TYG and TYE as well as TYD and TYE dilutors with respect to sperm motility percentage in both the breeds. Sperm motility was significantly higher in TYD and TYG dilutors than TYE dilutor for both breeds. No significant difference in sperm motility percentage noted in TYG and TYD dilutors in both breeds.
5.5.1.2 Sperm motility percentage at post-freezing (24 hours) stage

The average sperm motility percentage of semen at post-freezing (24 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 77.22±2.34, 78.38±2.04, 72.22±1.85 and Sirohi bucks it was 67.77±1.11, 72.76±1.02 and 64.43±2.04 respectively.

There was a significant difference (P<0.05) between two the breeds of bucks with respect to sperm motility percentage. Sperm motility was significantly higher in Osmanabadi than Sirohi bucks at 24 hours post-freezing stage.

There was a significant difference across different dilutors with respect to sperm motility percentage at post-freezing (24 hours). Stage

The results further indicated that in Osmanabadi bucks semen there was a significant difference between TYG- TYE and TYD-TYE dilutors with respect to sperm motility percentage at post freezing (24 hours) stage . Sperm motility was significantly higher in TYD and TYG than TYE dilutor. There was no significant difference in sperm motility percentage in TYG-TYD in Osmanabadi bucks

In Sirohi bucks semen the sperm motility was higher in TYD as compared to TYG and TYE dilutor in present study.

In both the breeds TYD dilutor shows better sperm motility, this indicates that DMSO was better cryo-protectant for preserving buck semen motility post-freezing.

5.5.1.3 Sperm motility percentage at post-freezing (72 hours) stage

The average sperm motility percentage of semen at post-freezing (72 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 73.89±2.00, 75.00±1.70 and 71.11±1.11 and for Sirohi bucks it was 67.23±1.02, 72.20±0.70 and 61.66±1.13 respectively.

There was a significant difference (P<0.05) between the two breeds of bucks with respect to post-freezing (72 hours) sperm motility percentage in TYG and TYE dilutor. The sperm motility was significantly higher in Osmanabadi than Sirohi bucks in
TYG and TYE dilutors. The sperm motility in TYD dilutor was not significantly different in both breeds indicating good sperm viability in TYD dilutor.

There was a significant difference (P<0.05) across different dilutors with respect to post-freezing (72 hours) sperm motility percentage.

The results further indicated that in Osmanabadi bucks no significant difference in sperm motility was observed across three dilutors.

In Sirohi bucks there was a significant difference between TYG and TYD, TYD and TYE as well as TYG and TYE dilutors with respect to post-freezing (72 hours) sperm motility percentage. The sperm motility reduced significantly in TYE than TYG and TYD dilutor. This indicates that ethylene glycol was not providing optimum cryo-protecting environment to bucks spermatozoa at post-freezing stage. The sperm motility was also significantly low in TYG as compared to TYD dilutor.

5.5.1.4 Sperm motility percentage in TYG dilutor

The average sperm motility percentage of semen in TYG dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 82.22±2.04, 77.22±2.34 and 73.89±2.00 and Sirohi bucks it was 71.66±1.42, 67.77±1.11 and 67.23±1.02 respectively.

There was a significant difference (P<0.05) between two the breeds of bucks with respect to sperm motility percentage in TYG dilutor at pre freezing and post freezing 24 and 72 hours stages. Sperm motility was significantly higher Osmanabadi than Sirohi bucks at all three stages of freezing in TYG dilutor.

There was a significant difference (P<0.05) across different time interval and temperatures with respect sperm motility percentage in TYG diluter.

In Osmanabadi bucks semen there was a significant difference between pre-freezing and 24 hours post-freezing, pre-freezing and 72 hours post-freezing sperm motility percent. Sperm motility was significantly reduced from pre-freezing to post freezing (24 hours) stage in Osmanabadi bucks.

In Sirohi bucks no significant difference in sperm motility was noted between pre-freezing and post freezing (24 hours) stage, but sperm motility was significantly reduced at 72 hours post freezing as compared to pre freezing stage.
As there was no significant difference noted in sperm motility percentage between post-freezing 24 and 72 hours for both breeds we can conclude that sperm motility was stable within normal limits in TYG dilutor after freezing of semen.

5.5.1.5 Sperm motility percentage in TYD dilutor

The average sperm motility percentage of semen in TYD dilutors at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 83.33±2.11, 78.38±2.04 and 75.00±1.85 and for Sirohi bucks it was 74.45±1.64, 72.76±1.02, and 72.20±2.04 respectively.

There was a significant difference (P<0.05) between the two breeds of bucks with respect to sperm motility percentage at pre-freezing and post freezing (24 and 72 hours) stages. Significantly higher sperm motility observed was in Osmanabadi than Sirohi bucks at pre-freezing than post freezing (24 hours) stage. No significant difference in sperm motility observed at post freezing (72 hours) stage in both the breeds.

There was a significant difference (P<0.05) across different time interval and temperature with respect to post thaw sperm motility in TYD dilutor.

The results further indicated that In Osmanabadi bucks there was a significant difference between pre-freezing and post-freezing (24 hours) stage as well as pre-freezing and post-freezing (72 hours) stage with respect to sperm motility percentage in TYD dilutor. Sperm motility significantly reduced at post freezing (24 hours) and post freezing (72 hours) stage than pre-freezing stage. Sperm motility was not significantly reduced from post freezing (24 hours) stage to post freezing (72 hours) stage.

In Sirohi bucks no significant difference noted in sperm motility percentage between different time interval and temperature in TYD dilutor.

There was no significant difference noted in sperm motility percentage between post-freezing (24 hours) and post freezing (72 hours) stage in both breeds we can conclude that sperm motility was stable in TYD dilutor after freezing.

5.5.1.6 Sperm motility percentage in TYE dilutor
The average sperm motility percentage in TYE dilutors at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 77.78±2.67, 72.22±1.85 and 71.11±1.11 and for Sirohi bucks it was 66.65±1.72, 64.43±2.04, and 61.66±1.13 respectively.

There was a significant difference (P<0.05) between two breeds of bucks with respect to sperm motility percentage at pre freezing and post freezing(24 and 72 hours) stages in TYE dilutor. Significantly higher sperm motility percentage observed was in Osmanabadi than Sirohi bucks.

There was a significant difference (P<0.05) across different dilutors with respect to sperm motility percentage in TYE dilutor.

The results further indicated that in Osmanabadi bucks semen there was a significant difference between pre-freezing and post-freezing (24 and 72 hours) sperm motility percentage in TYE dilutor. Sperm motility significantly reduced at 24 and 72 hours post-freezing than pre-freezing stage. No significant reduction in sperm motility noted between post-freezing stages. It indicates that sperm motility was stable post-freezing for Osmanabadi breed semen in TYE dilutor.

In Sirohi bucks semen there was no significant difference between pre-freezing and post-freezing (24 hours) as well as 24 hours post freezing and 72 hours post freezing sperm motility percentage in TYE dilutor. There was significant reduction in sperm motility was observed from pre freezing to post freezing (72 hours) stage.

5.5.2 Live sperm percentage

The live sperm percentage at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks semen was recorded as below.
5.5.2.1 Live sperm percentage at pre-freezing (5°C) stage

The average live sperm percentage of semen at pre-freezing (5°C) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 81.99±0.51, 82.94±0.76 and 78.55±1.07 and for Sirohi bucks it was 80.44±1.27, 81.66±1.25 and 79.88±1.24 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to live sperm percentage.

The results further indicated that in Osmanabadi bucks semen there was a significant difference between TYG and TYE as well as TYD and TYE dilutors with respect to live sperm percentage. Live sperm percentage was significantly higher in TYG and TYD than TYE dilutor. No significant difference in live sperm percentage was noted in TYG and TYD dilutors.

In Sirohi bucks semen no significant difference in live sperm percentage was noted in TYG and TYD, TYG and TYE as well as TYD and TYE dilutors.

5.5.2.2 Live sperm percentage at post-freezing (24 hours) stage

The average live sperm percentage in semen at post-freezing (24 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 78.55±0.70, 78.77±1.52 and 75.66±0.95 and for Sirohi bucks it was 78.77±1.38, 79.66±1.36 and 76.56±1.68 respectively.

There was no significant difference (P>0.05) observed between the two breeds as well as across different dilutors with respect to live sperm percentage. Live sperm percentage was non-significantly higher in Sirohi than Osmanabadi bucks at post-freezing (24 hours) stage.
5.5.2.3 Live sperm percentage at post-freezing (72 hours) stage

The average live sperm percentage of semen at post-freezing (72 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 77.83 ± 0.84, 77.50 ± 0.75 and 74.05 ± 0.52 and for Sirohi bucks it was 77.61 ± 1.29, 78.22 ± 1.41 and 74.72 ± 1.66 respectively.

There was no significant difference (P>0.05) between the two breed of bucks with respect to post-freezing (72 hours) live sperm percentage.

There was a significant difference (P<0.05) across different dilutors with respect to live sperm percentage.

The results further indicated that in Osmanabadi and Sirohi bucks semen there was a significant difference between dilutors TYG and TYE as well as TYD and TYE dilutors at post-freezing (72 hrs) stage with respect to live sperm percentage. Significantly higher live sperm percentage noted in TYG and TYD than TYE dilutors. There was no significant difference in TYG and TYD dilutors with respect to live sperm percentage. This indicates that glycerol and DMSO were better than ethylene glycol for preserving sperm viability in Osmanabadi and Sirohi bucks semen at post-freezing (72 hours) stage.

5.5.2.4 Live sperm percentage TYG dilutor

The average live sperm percentage of semen in TYG dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 81.99±0.51, 78.55±0.69 and 77.83±0.84 and for Sirohi bucks it was 80.44±1.26 79.66±1.38, 78.22±1.29 respectively.

There was no significant difference (P>0.05) with respect to live sperm percentage at pre and post freezing stages in both the breeds.

There was a significant difference (P<0.05) across different time interval and temperatures with respect to live sperm percentage in TYG dilutor.

The results further also indicated that in Osmanabadi bucks there was a significant difference between pre-freezing and 24 hours post-freezing as well as pre-freezing and 72 hours post-freezing stage in TYG dilutor with respect to live sperm
percentage. Live sperm percent reduced significantly from pre freezing to post freezing (24 and 72 hours) stage in Osmanabadi bucks semen.

In Sirohi bucks semen there was no significant reduction in live sperm percent from pre freezing to post freezing (24 and 72 hours) stage.

As there was no significant difference noted in live sperm percentage between post-freezing 24 and 72 hours in both the breeds we can conclude that sperm viability was stable in TYG dilutor after freezing.

5.5.2.5 Live sperm percentage TYD dilutor

The average live sperm percentage of semen in TYD dilutors at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 82.94±0.76, 78.77±1.52 and 77.50±0.75 and for Sirohi bucks it was 81.66±1.25, 79.66±1.36 and 78.22±1.41 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to live sperm percentage in TYD dilutor at different time interval and temperature.

There was a significant difference (P<0.05) across different time interval and temperature with respect to live sperm percentage in TYD dilutor.

The results further indicated that in Osmanabadi bucks semen there was a significant difference between pre-freezing and post-freezing (24 hours) as well as pre-freezing and post freezing (72 hours) stage with respect to live sperm percentage in TYD dilutor. Live sperm percentage was significantly reduced at post freezing 24 and 72 hours than pre-freezing stage.

In Sirohi bucks no significant difference noted in live sperm percentage in TYD dilutor at different time interval and temperature except pre freezing to post freezing (72 hours) stage. Significant reduction in live sperm percent was recorded from pre freezing to post freezing (72 hours) stage.

As there was, no significant difference noted between 24 and 72 hours post-freezing stage in both breeds with respect to live sperm percentage that indicates that sperm viability was not affected after 24 hours of freezing. It means that sperm viability was better preserved in TYD dilutor for both the breeds.
5.5.2.6 Live sperm percentage TYE dilutor

The average live sperm percentage in TYE dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 78.55 ± 1.07, 75.66±0.95 and 74.05±0.52 and for Sirohi bucks it was 79.88±1.24, 76.55±1.68 and 74.72±1.65 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to live sperm percentage in TYE dilutor.

There was a significant difference (P<0.05) across different time interval and temperature with respect to live sperm percentage in TYE dilutor.

The results further indicated that in Osmanabadi bucks semen there was a significant difference between pre-freezing and post freezing (72 hours) stage with respect to live sperm percentage in TYE dilutor. Live sperm percentage significantly reduced at post freezing (72 hours) stage than pre freezing stage. No significant difference was noted between pre freezing and post freezing (24 hours) as well as post freezing (24 hours) and post freezing (72 hours) stage.

In Sirohi bucks semen there was significant difference between pre-freezing and 24 hours post-freezing as well as pre-freezing and 72 hours post-freezing live sperm percent in TYE dilutor. Live sperm percentage significantly reduced post freezing (24 and 72 hours) than pre freezing stage.

In both breeds, no significant difference noted between 24 and 72 hours post-freezing stages. This indicates stable sperm viability after freezing.

5.5.3 Abnormal sperm percentage

The abnormal sperm percentage at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks semen was recorded as below.
5.5.3.1 Abnormal sperm percentage at pre-freezing (5\(^0\)C) stage

The average abnormal sperm percentage of semen at pre-freezing (5\(^0\)C) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 16.60±0.45, 16.11±0.49 and 17.88±0.73 and for Sirohi bucks it was 14.33±0.73, 15.72±0.95 and 16.61±1.03 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to abnormal sperm percentage except TYG dilutor. Significantly higher abnormal sperm percentage noted in Osmanabadi than Sirohi bucks at pre-freezing stage in TYG dilutor.

There was no significant difference (P<0.01) across different dilutors with respect to abnormal sperm percentage except TYG-TYE in Sirohi bucks. Significantly higher percentage of abnormal spermatozoa was noted in TYE than TYG dilutor in Sirohi bucks.

As there was no significant difference noted in sperm abnormality in different dilutors we can conclude that conditions in dilutors were optimum to preserve sperm morphology at pre-freezing stage.

5.5.3.2 Abnormal sperm percentage at post-freezing (24 hours) stage

The average abnormal sperm percentage of semen at post-freezing (24 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 18.61±0.52, 17.50±0.65 and 19.77±0.31 and for Sirohi bucks it was 17.55±1.05, 18.05±1.14 and 19.99±1.20 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to abnormal sperm percent.

There was significant (P<0.05) difference across different dilutors with respect to abnormal sperm percentage at post-freezing (24 hours) stage.

The results further indicated that in Osmanabadi bucks semen there was significant difference between TYD and TYE dilutors with respects to abnormal sperm percent. Significantly higher abnormal sperm percent was noted in TYE than TYD.
dilutor. There was no significant difference in TYG and TYD as well as TYG and TYE dilutors with respect to abnormal sperm percent at post freezing (24 hours) stage.

In Sirohi bucks semen significant difference was observed in sperm abnormality between TYD and TYE as well as TYG and TYE dilutors. Significantly higher sperm abnormalities were recorded in TYE dilutor as compared to TYG and TYD dilutors.

5.5.3.3 Abnormal sperm percentage at post-freezing (72 hours) stage

The average abnormal sperm percentage of semen at post-freezing (72 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 20.22 ± 0.52, 17.88 ± 0.65 and 22.22 ± 0.31 and for Sirohi bucks it was 19.72 ± 1.05, 19.72 ± 1.14 and 23.38 ± 1.20 respectively.

There was no significant difference (P>0.05) between the two breed with respect to abnormal sperm percentage at post-freezing (72 hours) stage. The results further indicated that there was a significant difference (P<0.05) across different dilutors with respect to abnormal sperm percentage.

In Osmanabadi bucks semen there was significant difference between TYD and TYE as well as TYG and TYD dilutor with respect to abnormal sperm percentage at post-freezing (72 hours) stage. Abnormal sperm percent were significantly higher in TYE than TYD as well as in TYG than TYD dilutor, which indicates that DMSO was better cryoprotectant than ethylene glycol and glycerol for Osmanabadi bucks semen preservation. There was no significant difference in TYG and TYE dilutor with respect to abnormal sperm percent.

In Sirohi bucks semen there was significant difference between TYG and TYE as well as TYD and TYE dilutors with respect to abnormal sperm percent at post freezing (72 hours) stage. Significantly higher abnormal sperm percent recorded was in TYE than TYG and TYD dilutor which indicates DMSO and glycerol as better cryoprotectant than ethylene glycol for Sirohi bucks semen preservation. All the abnormalities observed in both the breeds were within the normal prescribed limit.
5.5.3.4 Abnormal sperm percentage in TYG dilutor

The average abnormal sperm percentage of semen in TYG dilutor at pre-freezing (5\(^0\)C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 16.60±0.45, 18.61±0.62 and 20.22±0.40 and Sirohi bucks it was 14.33±0.86, 17.55±1.23 and 19.72±1.53 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks at post freezing stages but there was significant difference (P<0.05) at pre-freezing stage with respect to abnormal sperm percentage in TYG dilutor. Osmanabadi bucks semen showed significantly higher sperm abnormalities than Sirohi bucks at pre freezing stage in TYG dilutor.

There was a significant difference (P<0.05) across different time interval and temperature with respect to abnormal sperm percentage in TYG dilutor.

The results further indicated that in Osmanabadi bucks semen there was a significant difference between pre-freezing and post freezing (72 hours) stage only with respect to abnormal sperm percent in TYG dilutor. Sperm abnormalities increased significantly at post freezing (72 hours) stage than pre freezing in Osmanabadi bucks. There was, no significant difference noted in abnormal sperm percentage between post-freezing 24 and 72 hours in Osmanabadi bucks, we could conclude that sperm abnormality was not increased due to freezing in TYG dilutor.

In Sirohi bucks semen there was significant difference between pre-freezing and 24 and 72 hours post-freezing as well as post freezing 24 and 72 hours stage in TYG dilutor with respect to abnormal sperm percentage. In Sirohi bucks although there was significant difference between post freezing 24 and 72 hours stage with respect to sperm abnormality we can observe that the sperm abnormality were within prescribed normal limit at post freezing (72 hours) stage.

5.5.3.5 Abnormal sperm percentage in TYD dilutor

The average abnormal sperm percentage of semen in TYD dilutors at pre-freezing (5\(^0\)C), post-freezing (24 hours) and post-freezing (72 hours) stages for
Osmanabadi bucks was 16.11±0.49, 17.50±0.76 and 17.88±0.84 and for Sirohi bucks it was 15.72±0.95, 18.05±1.35 and 19.72±1.27 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to abnormal sperm percentage at different time interval and temperatures in TYD dilutor.

There was a significant difference (P<0.05) across different time interval and temperatures with respect to abnormal sperm percentage in TYD dilutor.

The results further indicated that in Osmanabadi bucks semen there was a significant difference between pre-freezing and post freezing (72 hours) stage with respect to sperm abnormalities. Sperm abnormalities increased significantly at post freezing (72 hours) than pre freezing stage. But there was no significant increased in sperm abnormalities between 24 to 72 hours post freezing stage.

In Sirohi bucks semen there was a significant difference between pre-freezing and post freezing (24 and 72 hours) stage with respect to sperm abnormalities. Sperm abnormalities increased significantly at post freezing (24 and 72 hours) than pre freezing stage. But there was no significant increased in sperm abnormalities between 24 to 72 hours post freezing stage.

As there was no significant increase in sperm abnormalities from 24 to 72 hours post freezing stage in both the breeds we can opined that sperm morphology was not affected in TYD dilutor on frozen storage of semen.

**5.5.3.6 Abnormal sperm percentage in TYE dilutor**

The average abnormal sperm percentage in TYE dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 17.88±0.73, 19.77±0.36 and 22.22±0.98 and for Sirohi bucks it was 16.61±1.03, 19.99±1.42 and 23.38±2.04 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to abnormal sperm percentage at different time interval and temperature in TYE dilutor.

There was a significant difference (P<0.05) across different time interval and temperature with respect to abnormal sperm percentage in TYE dilutor.

The results further indicated that in Osmanabadi bucks semen there was a significant difference with respect to abnormal sperm percentage between pre-
freezing and post-freezing (72 hours) stage in TYE dilutor. Sperm abnormality increased significantly at post freezing (72 hours) than pre freezing stage. No significant difference was noted between 24 and 72 hours post-freezing indicates that sperm abnormality was not increased on frozen storage.

In Sirohi bucks semen there was a significant difference with respect to abnormal sperm percentage between pre-freezing and post-freezing (24 hours) stage as well as pre-freezing and post-freezing (72 hours) stages in TYE dilutor. Sperm abnormality increased significantly at post freezing (24 and 72 hours) than pre freezing stage. Significant difference in abnormal sperm percentage was also noted between 24 and 72 hours post-freezing indicates that sperm abnormality was increasing as freezing time progressed. This indicates that TYE dilutor was not effective for protecting sperm morphology post freezing in Sirohi breed.

5.5.4 Plasma membrane integrity of spermatozoa (HOST)

The Plasma membrane integrity sperm percentage at different freezing stages in different dilutors of Osmanabadi and Sirohi bucks detected by Hypo-osmotic swelling test (HOST) was recorded as below.

5.5.4.1 Plasma membrane integrity of spermatozoa at pre-freezing (5°C) stage (HOST).

The average hypo-osmotic swelling sperm percentage of semen at pre-freezing (5°C) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 72.50±1.03, 74.22±1.03 and 72.05±2.54 and for Sirohi bucks it was 71.00±1.15, 72.11±1.15 and 67.83±0.62 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to HOS sperm percentage in TYG and TYD dilutors but significantly higher HOS sperm percent was noted in TYE dilutor for Osmanabadi than Sirohi bucks.
In Osmanabadi bucks semen there was no significant difference (P>0.05) across different dilutors with respect to HOS sperm percentage.

In Sirohi bucks semen there was significant difference between TYD and TYE as well as TYG and TYE dilutors. Significantly higher plasma membrane integrity observed in Sirohi bucks was in TYD and TYG than TYE dilutor at pre-freezing stage.

5.5.4.2 Plasma membrane integrity of spermatozoa at post-freezing (24 hours) stage (HOST).

The average hypo-osmotic swelling sperm percentage of semen at post-freezing (24 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 68.83±1.95, 72.66±1.78 and 68.72±1.46 and Sirohi bucks it was 68.83±0.59, 70.11±0.84 and 66.50±0.76 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks but there was a significant difference (P<0.05) across different dilutors with respect to HOS positive sperm percentage.

The results further indicated that in Osmanabadi bucks semen there was a significant difference between TYG and TYD as well as TYD and TYE dilutors. Significantly higher plasma membrane integrity noted in TYD than TYG and TYE dilutors.

In Sirohi bucks semen there was significant difference between TYD-TYE dilutors with respect to HOS sperm percentage at post-freezing (24 hours) stage. Significantly higher plasma membrane integrity observed was in TYD than TYE dilutor. There was no significant difference between TYG and TYD as well as TYG and TYE dilutors with respect to plasma membrane integrity.
5.5.4.3 Plasma membrane integrity of spermatozoa at post-freezing (72 hours) stage (HOST)

The average hypo-osmotic swelling sperm percentage of semen at post-freezing (72 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 67.27±1.16, 70.16±1.42 and 67.83±1.23 and for Sirohi bucks it was 66.33±0.72, 67.50±1.41 and 63.66±1.10 respectively.

There was a significant difference (P<0.05) between the two breeds of bucks with respect to HOS sperm percentage in TYD and TYE dilutors. Plasma membrane integrity was significantly higher in Osmanabadi than Sirohi bucks in TYE dilutors at post freezing (72 hours) stage.

There was a significant difference (P<0.05) across different dilutors with respect to HOS sperm percentage.

The results further indicated that in Osmanabadi bucks semen there was no significant difference across different dilutors with respect to plasma membrane integrity at post freezing (72 hours) stage.

In Sirohi bucks semen there was significant difference between TYD and TYE dilutor with respect to HOS sperm percentage at post freezing (72 hours) stage. Significantly, higher percentage of plasma membrane integrity observed was in TYD than TYE dilutors.

In both the breeds higher plasma membrane integrity observed was in TYD dilutor which indicates that DMSO was more effective cryoprotectant than ethylene glycol and glycerol in preserving sperm membrane integrity on frozen storage of Osmanabadi and Sirohi bucks semen.
5.5.4.4 Plasma membrane integrity (HOST) of spermatozoa in TYG dilutor

The average hypo-osmotic swelling sperm percentage in TYG dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 72.50±1.03, 68.83±1.95 and 67.27±1.16 and for Sirohi bucks it was 71.00±1.14, 68.83±0.59, and 66.33±0.72 respectively.

In TYG dilutor there was no significant difference (P>0.05) between the two breeds of bucks but there was a significant difference (P<0.05) across different time interval and temperatures with respect to hypo-osmotic swelling sperm percentage.

The results further indicated that in Osmanabadi bucks semen there was a significant difference between pre-freezing and post freezing (24 hours) as well as pre-freezing and post freezing (72 hours) stage with respect to hypo-osmotic swelling sperm percentage. Significant reduction in plasma membrane integrity was recorded at post freezing (24 and 72 hours) stage as compared to pre freezing stage.

In Sirohi bucks semen there was significant difference between pre-freezing and post freezing (72 hours) stage with respect to hypo-osmotic swelling sperm percentage. Significant reduction in plasma membrane integrity recorded was at post freezing (72 hours) stage as compared to pre freezing stage.

Plasma membrane integrity was not significantly reduced between 24 to 72 hours post freezing in both breeds. This indicates that plasma membrane integrity was not damaged once the spermatozoa were frozen in TYG dilutor. It indicates that glycerol was good cryoprotectant and was able to maintain sperm membrane integrity after freezing of semen.

5.5.4.5 Plasma membrane integrity (HOST) of spermatozoa in TYD dilutor

The average hypo-osmotic swelling sperm percentage in TYD dilutors at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 74.22±1.02, 72.67±1.78 and 70.17±1.42 and for Sirohi bucks it was 72.11±0.89, 70.11±0.84 and 67.50±1.41 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to hypo-osmotic swelling sperm percentage at different time interval and temperatures in TYD dilutor.
There was significant difference across different time interval and temperatures with respect to hypo-osmotic swelling sperm percentage in TYD dilutor.

The results further indicated that in both the breeds there was significant difference between pre-freezing and post-freezing (24 and 72 hours) stage with respect to hypo-osmotic swelling sperm percentage in TYD dilutor. Significant reduction in hypo-osmotic swelling sperm percentage was noted between pre freezing and post freezing (24 and 72 hours) stage in TYD dilutor. But there was no significant reduction in hypo-osmotic swelling sperm percentage between post freezing 24 and 72 hours, which indicates good cryoprotecting ability of DMSO in both the breeds. Plasma membrane integrity of spermatozoa of both the breeds was maintained in TYD dilutor on frozen storage.

5.5.4.6 Plasma membrane integrity (HOST) of spermatozoa in TYE dilutor

The average sperm plasma membrane integrity percentage detected by hypo-osmotic swelling test (HOST) in TYE dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 72.05±2.54, 68.72±1.46 and 67.83±1.23 and for Sirohi bucks it was 67.83±0.62, 66.50±0.76 and 63.66±1.10 respectively.

There was a significant difference (P<0.05) between the two breeds of bucks with respect to sperm plasma membrane integrity percentage in TYD dilutor at pre-freezing and post freezing (72 hours) stage in TYE dilutor. Significantly higher plasma membrane integrity observed was in Osmanabadi bucks than Sirohi bucks at pre freezing and post freezing (72 hours) stage.

There was a significant difference (P<0.05) across different time and temperature interval with respect to sperm plasma membrane integrity in TYE dilutor.
The results further indicated that in Osmanabadi bucks semen there was significant difference between pre-freezing and post-freezing (24 and 72 hours) stage with respect to sperm plasma membrane integrity detected by HOST. Significant reduction in sperm plasma membrane integrity was observed from pre-freezing to post-freezing (24 and 72 hours) stage, but plasma membrane integrity was not significantly reduced between 24 and 72 hours post-freezing stage.

In Sirohi bucks semen significant difference in sperm plasma membrane integrity noted was between pre-freezing and post freezing (72 hours) stage. Plasma membrane integrity was significantly reduced from pre-freezing to post freezing (72 hours) stage, but plasma membrane integrity was not significantly reduced between 24 and 72 hours post-freezing stage.

As there was no significant reduction in plasma membrane integrity from post-freezing 24 to 72 hours stage indicates that plasma membrane integrity was maintained post freezing in TYE dilutor for both the breeds.

5.5.5 Acrosome intactness of spermatozoa (Giemsa stain)

The acrosome intact sperm percentage at different freezing stages in different dilutors of Osmanabadi and Sirohi bucks detected by Giemsa stain was recorded as below.

5.5.5.1 Acrosome intactness of spermatozoa at pre-freezing (5°C) stage

The average Acrosome intact sperm percentage of semen at pre-freezing (5°C) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 86.89±1.00, 88.50±0.90 and 85.99±0.98 and for Sirohi bucks it was 85.22±1.53, 87.38±1.44 and 85.05±1.63, respectively.
There was no significant difference (P>0.05) between the two breeds of bucks and across the different dilutor with respect to acrosome intact sperm percentage at pre-freezing stage (5°C).

The literature pertaining to buck semen for acrosome intactness at pre freezing stage was limited; hence the comparison was also made with other species.

5.5.5.2 Acrosome intactness of spermatozoa at post freezing (24 hours) stage

The average Acrosome intact sperm percentage of semen at post-freezing (24 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 85.00±1.25, 86.94±0.94 and 84.39±0.78 and for Sirohi bucks it was 82.89±0.73, 86.44±1.23 and 83.11±1.07 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks but there was a significant difference (P<0.05) across different dilutors with respect to acrosome intact sperm percentage with Geimsa stain.

The results further indicated that in Osmanabadi bucks semen there was no significant difference between different dilutors with respect to acrosome intact sperm percentage at post freezing (24 hours) stage.

In Sirohi bucks semen there was significant difference between TYG and TYD as well as TYD-TYE dilutors with respect to acrosome intact sperm percentage at post-freezing (24 hours) stage. Significantly higher acrosome intactness observed was in TYD dilutor in Sirohi bucks as compared to TYG and TYE dilutors. No significant difference observed across TYG and TYE dilutors.

5.5.5.3 Acrosome intactness of spermatozoa at post freezing (72 hours) stage

The average Acrosome intact sperm percentage of semen at post-freezing (72 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 83.61±1.30, 85.11±1.21 and 83.27±1.20 and for Sirohi bucks it was 83.05±1.51, 86.22±1.20 and 82.55±1.34 respectively.
There was no significant difference (P>0.05) between the two breeds of bucks with respect to acrosome intact sperm percent in different dilutors at post freezing (72 hours) stage.

The results further indicated that in Osmanabadi bucks semen there was no significant difference between different dilutors with respect to acrosome intact sperm percentage at post freezing (24 hours) stage.

In Sirohi bucks semen there was significant difference between TYG and TYD as well as TYD-TYE dilutors with respect to acrosome intact sperm percentage at post-freezing (24 hours) stage. Significantly higher acrosome intactness observed was in TYD dilutor in Sirohi bucks as compared to TYG and TYE dilutors. No significant difference observed across TYG and TYE dilutors.

5.5.5.4 Acrosome intactness of spermatozoa in TYG dilutor

The average acrosome intactness of spermatozoa in TYG dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 86.89±1.00, 85.00±1.48 and 83.61±1.30 and for Sirohi bucks it was 85.22±1.53, 82.89±0.86 and 83.05±1.51 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to acrosome intact sperm percentage across different time interval and temperature in TYE dilutor.

There was significant difference (P>0.05) across different time interval and temperatures with respect to acrosome intactness in TYG dilutor.

The result further indicated that in Osmanabadi bucks semen there was significant reduction in acrosome intact sperm percent from pre freezing to post freezing (72 hours) stage, but there was no significant reduction in acrosome intactness observed between post freezing 24 and 72 hours stage in TYG dilutor.

In Sirohi bucks semen there was no significant difference across different time interval and temperature with respect to acrosome intact sperm percent in TYG dilutor. In both the breeds as there was no significant reduction in acrosome intactness between post freezing 24 and 72 hours stage it can be concluded that glycerol was effective in maintaining acrosome intactness of bucks spermatozoa.
5.5.5.5 Acrosome intactness of spermatozoa in TYD dilutor

The average acrosome intactness of spermatozoa detected by Geimsa stain in TYD dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 88.50±0.90, 86.94±1.12 and 85.11±1.21 % and for Sirohi bucks it was 87.38±1.44, 86.44±1.46 and 86.22 ± 1.20 % respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to acrosome intact sperm percentage across different time interval and temperature in TYD dilutor.

There was significant difference (P<0.05) across different time interval and temperatures with respect to acrosome intactness in TYD dilutor.

The result further indicated that in Osmanabadi bucks semen there was significant reduction in acrosome intact sperm percent from pre freezing to post freezing (72 hours) stage, but there was no significant reduction in acrosome intactness observed between post freezing 24 and 72 hours stage in TYD dilutor.

In Sirohi bucks semen there was no significant difference across different time interval and temperature with respect to acrosome intact sperm percent in TYD dilutor.

In both the breeds as there was no significant reduction in acrosome intactness between post freezing 24 and 72 hours stage it can be concluded that DMSO was effective in maintaining acrosome intactness of bucks spermatozoa.

5.5.5.6 Acrosome intactness of spermatozoa in TYE dilutor

The average acrosome intactness of spermatozoa by Geimsa stain in TYE dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 85.99±0.98, 84.39±0.92, 83.27±1.20 % and for Sirohi bucks it was 85.05±1.63, 83.11±1.26, 82.55±1.34% respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to acrosome intact sperm percentage across different time interval and temperature in TYE dilutor.
There was significant difference (P<0.05) across different time interval and temperatures with respect to acrosome intactness in TYE dilutor.

The result further indicated that in Osmanabadi bucks semen there was significant reduction in acrosome intact sperm percent from pre freezing to post freezing (72 hours) stage, but there was no significant reduction in acrosome intactness observed between post freezing 24 and 72 hours stage in TYE dilutor.

In Sirohi bucks semen there was no significant difference across different time interval and temperature with respect to acrosome intact sperm percent in TYE dilutor. In both the breeds as there was no significant reduction in acrosome intactness between post freezing 24 and 72 hours stage it can be concluded that ethylene glycol was effective in maintaining acrosome intactness of bucks spermatozoa.

5.6.6 DNA damaged spermatozoa (Acridine Orange test)

The DNA damaged sperm percentage at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks detected by Acridine Orange test was recorded as below.

5.5.6.1 DNA damaged spermatozoa at pre-freezing (5°C) stage (Acridine Orange test).

There was no DNA damaged sperm detected by Acridine orange test at pre-freezing (5°C) stage in TYG, TYD and TYE dilutors for Osmanabadi and Sirohi bucks.

5.5.6.2 DNA damaged spermatozoa at post-freezing (24 hours) stage (Acridine Orange test).

The average DNA damaged sperm percentage detected by Acridine orange test in semen at post-freezing (24 hours) stage in TYG, TYD and TYE dilutors for
Osmanabadi bucks was 5.66±0.95, 4.83±1.02 and 8.15±0.85 and for Sirohi bucks it was 6.55±0.74, 5.41±1.40 and 5.77±0.55 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks in TYG and TYD dilutors with respect to DNA damaged sperm percent but there was significantly higher (P<0.05) DNA damaged sperm detected in Osmanabadi than Sirohi bucks in TYE dilutor at post freezing (24 hours) stage.

There was significant difference between DNA damaged sperm percent across different dilutors at post freezing (24 hours) stage.

The results further indicated that in Osmanabadi bucks semen there was significant difference between TYG and TYE as well as TYD and TYE dilutors with respect to DNA damaged sperm percent at post freezing stage (24 hours) stage. Significantly higher DNA damaged sperm percentage observed was in TYE than TYG and TYD dilutors. No significant difference between TYG and TYD dilutors with respect to DNA damaged sperm percent at post freezing stage (24 hours) stage.

In Sirohi bucks semen there was no significant difference observed across different dilutors at post freezing (24 hours) stage with respect to DNA damaged sperm percent.

5.5.6.3 DNA damaged spermatozoa at post-freezing (72 hours) stage (Acridine Orange test).

The average DNA damaged sperm percentage detected by Acridine orange test in semen at post-freezing (72 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 6.44±1.05, 6.38±1.01 and 8.94±0.58 and for Sirohi bucks it was 7.89±0.82, 7.77±1.76 and 8.94±0.70 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to DNA damaged sperm percent at post freezing (72 hours) stage in different dilutors.

There was significant difference between DNA damaged sperm percent across different dilutors at post freezing (72 hours) stage.

The results further indicated that in Osmanabadi bucks semen there was significant difference between TYG and TYE as well as TYD and TYE dilutors with
respect to DNA damaged sperm percent at post freezing stage (72 hours) stage. Significantly higher DNA damaged sperm percentage observed was in TYE than TYG and TYD dilutors. No significant difference between TYG and TYD dilutors with respect to DNA damaged sperm percent at post freezing stage (72 hours) stage.

In Sirohi bucks semen there was no significant difference observed across different dilutors at post freezing (72 hours) stage with respect to DNA damaged sperm percent.

5.5.6.4 DNA damaged spermatozoa in TYG dilutor (Acridine Orange test)

The average DNA damaged spermatozoa percentage detected by Acridine Orange (AO) test in TYG dilutor at Pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 0.00, 5.66±1.12, and 6.44±1.05 and for Sirohi bucks it was 0.00, 6.55±0.88 and 7.89±0.70 respectively.

There was no significant difference (P>0.05) observed at pre-freezing, 24 and 72 hours post-freezing with respect to DNA damaged sperm percentage detected by AO test in TYG dilutor. DNA damaged sperm were detected only after freezing in TYG dilutor, it indicates that freezing causes DNA damage in sperm.

The DNA damaged sperm were detected after freezing in TYG dilutor but there was no significant increase in DNA damaged sperm percentage from 24 to 72 hours post freezing in both breeds, it indicates that sperm DNA damage was not increased significantly after freezing in TYG dilutor.

On scanning available literature, references regarding post-freezing DNA damage of spermatozoa especially in TYG dilutor not found so comparison could not be made.

5.5.6.5 DNA damaged spermatozoa in TYD dilutor (Acridine Orange test)

The average DNA damaged sperm percentage detected by Acridine orange (AO) test in TYD dilutors at pre-freezing (5°C), post-freezing (24 hours) and post-
freezing (72 hours) stages for Osmanabadi bucks was 0.00, 4.83±1.21 and 6.38±1.01 and for Sirohi bucks it was 0.00, 5.41±1.65 and 7.77±1.76 respectively.

There was no significant difference (P>0.05) observed at pre-freezing, 24 and 72 hours post-freezing with respect to DNA damaged sperm percentage detected by AO test in TYD dilutor. DNA damaged sperm were detected only after freezing in TYD dilutor, it indicates that freezing causes DNA damage in sperm.

The DNA damaged sperm were detected after freezing in TYD dilutor but there was no significant increase in DNA damaged sperm percentage from 24 to 72 hours post freezing in both breeds, it indicates that sperm DNA damage was not increased significantly after freezing in TYD dilutor.

5.5.6.6 DNA damaged spermatozoa in TYE dilutor (Acridine Orange test)

The average DNA damaged sperm percentage detected by Acridine orange (AO) test in TYE dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 00.00, 8.15±0.85 and 8.94±0.58 and for Sirohi bucks it was 00.00, 5.77±0.55 and 7.89±0.70 respectively.

There was significant difference (P<0.05) between the two breeds of bucks at post freezing (24 hours) stage with respect to DNA damaged sperm percent in TYE dilutor. Significantly higher DNA damaged sperm were detected at post freezing (24 hours) stage in Osmanabadi than Sirohi bucks.

The result further suggested that in Osmanabadi bucks semen there was no significant difference between post freezing 24 and 72 hours stage with respect to DNA damaged sperm percent in TYE dilutor, but in Sirohi bucks semen there was significant difference between 24 and 72 hours post freezing stage. Significantly higher DNA damaged sperm were detected at 72 hrs than 24 hrs post freezing in TYE dilutor in Sirohi bucks; which indicates DNA damage was increasing as time progressed post freezing in TYE dilutor which could be reason of low fertility.
5.5.7 DNA damaged spermatozoa percentage (Comet assay)

The DNA damaged spermatozoa percentage at different freezing stages indifferent dilutors in Osmanabadi and Sirohi bucks semen detected by Comet assay was recorded as below.

5.5.7.1 DNA damaged spermatozoa at pre-freezing (5°C) stage (Comet assay)

There was no DNA damage detected by Comet assay at pre-freezing (5°C) stage in TYG, TYD and TYE dilutors for Osmanabadi and Sirohi bucks.

5.5.7.2 DNA damaged spermatozoa at post-freezing(24 hours) stage (Comet assay)

The average DNA damage sperm percentage detected by Comet assay in semen at post-freezing (24 hours) stage in TYG, TYD and TYE dilutors for Osmanabadi bucks was 1.49±0.18, 1.38±0.30 and 1.28±0.16 and for Sirohi bucks it was 1.38±0.31, 1.15±0.26 and 1.43±0.18 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks as well as across different dilutors with respect to DNA damaged sperm percentage detected by Comet assay at post freezing (24 hours) stage.

5.5.7.3 DNA damaged spermatozoa at post-freezing(72 hours) stage (Comet assay)

The average DNA damaged sperm percentage detected by Comet assay in semen at post-freezing (72 hours) stage in TYG, TYD and TYE dilutors for
Osmanabadi bucks was 1.89 ± 0.20, 1.66±0.31, and 1.83 ± 0.20 and for Sirohi bucks it was 1.94 ± 0.29, 1.83±0.27 and 2.44±0.31 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks as well as across different dilutors with respect to DNA damage detected by Comet assay at post freezing (72 hours) stage.

5.5.7.4 DNA damaged spermatozoa in TYG diluter (Comet assay)

The average DNA damaged spermatozoa detected by Comet assay in TYG dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 00.00, 1.49 ± 0.18 and 1.89 ± 0.20 and for Sirohi bucks it was 00.00, 1.38 ± 0.36 and 1.94 ± 0.29 respectively.

There was no significant difference (P>0.05) observed in two breeds of bucks as well as at 24 and 72 hours post-freezing stage with respect to DNA damaged sperm percentage detected by Comet assay in TYG dilutor. DNA damaged sperm noted only after freezing in TYG dilutor, but the damage was not significantly increased at 72 hours post-freezing.

5.5.7.5 DNA damaged spermatozoa in TYD dilutor (Comet assay)

The average DNA damaged spermatozoa detected by Comet assay in TYD dilutors at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 0.00, 1.38±0.36, 1.66±0.31 and for Sirohi bucks it was 0.00, 1.15±0.31, 1.83±0.27 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks as well as there was no significant difference (P>0.05) across post freezing stages (24 and 72 hours) with respect to percent DNA damaged spermatozoa detected by Comet assay in TYD diluter. This indicates that however in TYD dilutor there was damage to sperm DNA on freezing it was not significantly increased after freezing.

5.5.7.6 DNA damaged spermatozoa in TYE dilutor (Comet assay)
The average DNA damaged spermatozoa percent detected by Comet assay in TYE dilutors at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 0.00, 1.28±0.20 and 1.83±0.20 and for Sirohi bucks it was 0.00, 1.43±0.20 and 2.44±0.31 respectively.

There was no significant difference (P>0.05) between the two breeds of bucks at pre-freezing and post freezing (24 and 72 hours) stage.

There was significant difference (P<0.05) across different time interval and temperatures with respect to DNA damaged spermatozoa detected by comet assay in TYE dilutor.

The results further indicated that in Osmanabadi bucks semen there was no significant difference between post-freezing (24 hours) and post freezing (72 hours) stage with respect to DNA damaged sperm percentage, which indicates DNA damage was not increasing as time progressed post freezing in TYE dilutor.

In Sirohi bucks semen there was significant difference between post freezing (24 hours) and post freezing (72 hours) stage with respect to DNA damaged sperm percentage. Significantly higher DNA damaged sperm percentage recorded was at post freezing (72 hours) than post-freezing (24 hours) stage; which indicates DNA damage was increasing as time progressed post freezing in TYE dilutor which could be reason of low fertility.

5.5.8 Glutamic – Oxaloacetic Transaminase (GOT) and Glutamic – Pyruvate Transaminase (GPT) activity

The Glutamic – Oxaloacetic Transaminase (GOT) and Glutamic – Pyruvate Transaminase (GPT) activity at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks semen was recorded as below.
5.5.8.1 GOT and GPT at pre-freezing (5°C) stage.

The average GOT at pre-freezing (5°C) stage in TYG, TYD, and TYE dilutors for Osmanabadi bucks was 110.30±8.69, 101.33±7.47, 112.22±8.01 IU/L and for Sirohi bucks it was 87.55±3.50, 86.16±3.50 and 94.05±4.91 IU/L respectively.

There was significant difference (P<0.05) between the two breeds of bucks with respect to GOT leakage except TYD dilutor. Significantly higher leakage of GOT was noted in TYG and TYE dilutors in Osmanabadi than Sirohi bucks.

There was no significant difference (P>0.05) across different dilutors with respect to GOT in semen of Osmanabadi and Sirohi bucks at pre-freezing stage. GOT release was not differing significantly in all three dilutors indicates that change in dilutors did not affect the GOT at pre-freeze stage of semen.

The average GPT at pre-freezing (5°C) stage in TYG, TYD, and TYE dilutors for Osmanabadi bucks was 20.06±1.12, 19.49±1.08 and 22.72±1.11 IU/L and for Sirohi bucks it was 20.11±0.89, 20.38±0.94 and 24.05±1.22 IU/L respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to GPT. GPT was not affected by breed difference.

There was significant difference (P<0.05) across different dilutors with respect to GPT.

The results further indicated that in both the breeds there was a significant difference between dilutors TYG-TYE, and TYD-TYE with respect to GPT in both breeds. GPT leakage was higher in TYE than TYG and TYD dilutor. There was no significant difference in GPT leakage in TYG and TYD dilutor. This indicates that sperm damage was higher in TYE than other two dilutors.
5.5.8.2 GOT and GPT at post-freezing (24 hours) stage.

At post-freezing stage (24 hours) the average GOT in TYG, TYD and TYE dilutors for Osmanabadi bucks was 116.33±8.16, 108.11±7.94 and 118.00±7.46 IU/L and for Sirohi bucks it was 98.44±5.22, 91.94±4.42 and 106.22±5.43 IU/L respectively.

There was a significant difference (P<0.05) between the two breeds of bucks with respect to GOT leakage in TYG and TYE dilutors. Significantly, higher GOT leakage noted was in TYG and TYE dilutors in Osmanabadi than Sirohi bucks at post-freezing (24 hours) stage. This indicates higher sperm damage in Osmanabadi bucks than Sirohi bucks.

There was no significant difference (P>0.05) across different dilutors with respect to GOT at post-freezing (24 hours) stage. This indicates that all three dilutors were providing optimum condition for spermatozoa with respect to GOT levels.

At post-freezing stage (24 hours) the average GPT in TYG, TYD and TYE dilutors for Osmanabadi bucks was 22.66±1.12, 21.72±1.03 and 25.05±0.65 IU/L and for Sirohi bucks it was 24.16±0.80, 24.77±0.84 and 28.33±0.70 IU/L respectively.

There was a significant difference (P<0.05) between the two breeds of bucks with respect to GPT in TYD and TYE dilutors. Significantly higher GPT was recorded in Sirohi than Osmanabadi bucks semen in TYD and TYE dilutors at post-freezing (24 and 72 hours) stage.

There was significant difference (P<0.05) across different dilutors with respect to GPT at post-freezing (24 hours) stage.

In Osmanabadi and Sirohi bucks semen there was significant difference in TYD and TYE dilutors with respect to GPT at post-freezing (24 hours) stage. Significantly higher GPT leakage noted was in TYE than TYD dilutor at post freezing (24 hours) stage in both the breeds. In Osmanabadi bucks no significant difference observed between TYG and TYD as well as TYG and TYE dilutor. In Sirohi bucks semen significant difference was also noted in TYG and TYE dilutors. Significantly higher GPT was noted in TYE than TYG dilutor indicates higher sperm damage in TYE than TYG dilutor.
### 5.5.8.3 GOT and GPT at post-freezing (72 hours) stage.

At post-freezing stage (72 hours) average GOT in TYG, TYD and TYE dilutors for Osmanabadi bucks was 119.72±7.75, 112.50±7.54 and 123.33±7.12 IU/L and for Sirohi bucks it was 102.50±4.96, 103.06±5.51 and 121.22±7.25 IU/L respectively.

At post-freezing (72 hours) stage there was no significant difference (P>0.05) between the two breeds of bucks in TYD and TYE dilutor, but there was significant difference (P<0.05) between two breeds of bucks in TYG dilutor. Significantly higher GOT was recorded in Osmanabadi than Sirohi bucks in TYG dilutor at post freezing (72 hours) stage.

The result further also indicated that there was significant leakage of GOT in both the breeds in TYG and TYE as well TYD and TYE dilutors. Significantly higher GOT was noted in TYG dilutor as compared to TYG and TYD dilutors at post freezing (72 hours) stage. There was no significant difference between TYG and TYD dilutor with respect to GOT in both the breeds.

At post-freezing stage (72 hours) average GPT in TYG, TYD and TYE dilutors for Osmanabadi bucks was 23.50±1.04, 22.89±1.24 and 26.33±0.81 IU/L and for Sirohi bucks it was 26.11±0.63, 27.16±0.91, 30.50±0.69 IU/L respectively.

There was a significant difference (P<0.05) between the two breeds of bucks with respect to GPT in all three dilutor at post freezing (72 hours) stage. GPT leakage was more in Sirohi than Osmanabadi bucks.

There was a significant difference (P<0.05) across different dilutors with respect to GPT at post-freezing (72 hours) stage.

The results further indicted that in both the breeds there was a significant difference between TYG and TYE as well as TYD and TYE dilutors with respect to GOT at post freezing (72 hours) stage. Significantly, higher GPT leakage noted was in TYE than TYD and TYG dilutors.

There was no significant difference between TYG and TYD dilutor in both the breeds bucks with respect to GPT at post freezing (72 hours) stage.

Significantly, higher GPT leakage noted was in TYE than TYD and TYG dilutors. In both the breeds indicates, that DMSO was better cryoprotectant than Ethylene glycol and glycerol for semen preservation of both breeds.
5.5.8.4 GOT and GPT in TYG dilutor.

The average GOT in TYG dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 110.30 ± 8.69, 116.33±8.16 and 119.72 ± 7.75 IU/L and for Sirohi bucks it was 87.55 ± 3.50, 98.44 ± 5.22 and 102.50±4.96 IU/l respectively.

In TYG dilutor there was a significant difference (P<0.05) between the two breeds of bucks. Significantly higher GOT leakage observed was in Osmanabadi than Sirohi bucks at all freezing stages in TYG dilutor.

There was no significant difference (P>0.05) across different time interval and temperatures with respect to GOT in TYG dilutor which indicates that GOT leakage is not significantly increased in TYG dilutor from pre-freezing to post-freezing. GOT leakage was more in Osmanabadi bucks than Sirohi bucks at post-freezing stage.

The average GPT in TYG dilutor at pre-freezing (5°C), post freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 20.06±1.12, 22.66±1.12, and 23.50±1.04 IU/l and Sirohi bucks it was 20.11±0.89, 24.16±0.80 and 26.11±063 IU/L respectively.

There was no significant difference (P>0.05) between the two breeds of bucks at Pre-freezing and post freezing (24 hours) stage but there was significant difference at post freezing (72 hours) stage with respect to GPT in TYG dilutor. Significantly, higher GPT observed was in Sirohi than Osmanabadi bucks at post freezing (72 hours) stage.

There was a significant difference (P<0.05) across different time interval and temperatures with respect to GPT in TYG dilutor.

The results further indicated that in both breeds there was a significant difference between pre-freezing and 24 hours post-freezing as well as pre-freezing and 72 hours post-freezing stages in TYG dilutor with respect to GPT. There was no significant difference between 24 and 72 hours post freezing stages with respect to GPT in TYG dilutor. This indicates significant higher leakage of GPT immediately after freezing, but leakage reduced subsequently in TYG dilutor.
**5.5.8.5 GOT and GPT in TYD dilutor.**

GOT in TYD dilutor at pre-freezing ($5^\circ\text{C}$), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was $101.33\pm 7.47$, $108.11\pm 7.94$ and $112.50\pm 7.54$ IU/L and for Sirohi bucks it was $86.16\pm 3.50$, $91.94\pm 4.42$ and $103.06\pm 5.51$ IU/L respectively.

There was no significant difference ($P>0.05$) between the two breeds of bucks with respect to GOT at pre freezing and post freezing (72 hours) stage in TYD dilutor. Significantly high GOT was noted in at post freezing (24 hours) stage in Osmanabadi bucks.

The results further indicated that in Osmanabadi bucks semen there was no significant difference ($P>0.05$) across different time interval and temperatures with respect to GOT in TYD dilutor. In Sirohi bucks semen there was significant difference between pre freezing and post freezing (72 hours) stage with respect to GOT leakage in TYD dilutor.

In both the breeds no significant difference in GOT was noted between post freezing 24 and 72 hours stage, which indicates sperm damage was not significantly increased post freezing in TYD dilutor.

The GPT in TYD dilutor at pre-freezing ($5^\circ\text{C}$), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was $19.49\pm 1.08$, $21.72\pm 1.03$ IU/L and $22.89\pm 1.24$ and for Sirohi bucks it was $20.38\pm 0.94$, $24.77\pm 0.84$ and $27.16\pm 0.91$ IU/L respectively.

There was a significant difference ($P<0.05$) between the two breeds of bucks with respect to GPT at post freezing (24 and 72 hours) stages. Sirohi bucks semen shows higher GPT leakage at post freezing (24 and 72 hours) stages than Osmanabadi bucks.

There was significant difference ($P<0.05$) across different time interval and temperatures with respect to GPT in TYD dilutor.

The results further indicated that in Osmanabadi bucks semen there was significant difference between pre-freezing and 72 hours post-freezing GPT. Significantly high GPT leakage was noted at post freezing (72 hours) stage as compared to pre freezing in TYD dilutor.
In Sirohi bucks semen significant GPT leakage was noted between pre freezing and post freezing (24 and 72 hours) stage in TYD dilutor.

In both the breeds there was no significant leakage of GPT between 24 and 72 hours post-freezing stage; which indicates that GPT leakage was not significantly increased in both the breeds post freezing.

5.5.8.6 GOT and GPT in TYE dilutor.

The average GOT in TYE dilutor at pre-freezing (5⁰C), post-freezing (24 hours) and post-freezing (72 hours) stage for Osmanabadi bucks was 112.22±8.01, 118.00±7.46 and 123.33±7.12 IU/L and for Sirohi bucks, it was 94.05±4.91, 106.22±5.43 and 121.22±7.25 IU/L respectively.

There was no significant difference (P>0.05) between post freezing (24 and 72 hours) stage with respect to GOT in two breeds of bucks in TYE dilutor.

There was a significant difference (P<0.05) across different time interval and temperatures with respect to GOT in TYE dilutor.

The results further indicated that there was a no significant difference (P>0.05) across different time interval and temperatures with respect to GOT in TYE dilutor in Osmanabadi bucks semen.

In Sirohi bucks semen there was a significant difference between pre-freezing and 72 hours post-freezing stage with respect to GOT in TYE dilutor. Significantly higher GOT was noted at post freezing (72 hours) stage than pre-freezing (5⁰C) stage in TYE dilutor.

In both the breeds of bucks semen no significant difference in GOT was noted between 24 and 72 hours post-freezing, it indicates leakage GOT after 24 hours of freezing was stable which indicates that sperm damage was not increased after 24 hours of freezing.

The average GPT in TYE dilutor at pre-freezing (5⁰C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 22.72±1.11, 25.05±0.65 and 26.33±0.81 IU/L and for Sirohi bucks it was 24.05±1.22, 28.33±0.70 and 30.50±0.69 IU/L respectively.
There was a significant difference between the two breeds of bucks at post freezing (24 and 72 hours) stage with respect to GPT in TYE dilutor. GPT was significantly higher at post freezing 24 and 72 hours in Sirohi than Osmanabadi bucks.

There was a significant difference (P<0.05) across different time interval and temperatures with respect to GPT in TYE dilutor.

The results further indicated that in Osmanabadi bucks semen there was a significant difference between pre-freezing and 72 hours post-freezing GPT in TYE dilutor. Significantly, increased GPT noted was at 72 hours post-freezing than pre-freezing stage in Osmanabadi bucks in TYE dilutor.

In Sirohi bucks semen, there was a significant difference between pre-freezing and 24 hours post freezing as well as pre-freezing and 72 hours post-freezing GPT in TYE dilutor. In both breeds, no significant difference in GPT was noted between 24 and 72 hours post-freezing, it indicates leakage GOT after 24 hours of freezing was not increased significantly; means that sperm damage was not increased significantly after 24 hours of freezing.

### 5.5.9 Alkaline phosphatase (AKP) and Acid phosphatase activity

The Alkaline phosphatase (AKP) and Acid phosphatase (ACP) activity at different freezing stages in different dilutors in Osmanabadi and Sirohi bucks semen was recorded as below.

#### 5.5.9.1 Alkaline phosphatase (AKP) and Acid phosphatase activity at pre-freezing (5°C) stage.

At pre-freezing (5°C) stage average AKP in TYG, TYD and TYE dilutors for Osmanabadi bucks was 1001.38±5.19, 1003.11±7.71 and 1008.76±6.02 IU/L and for Sirohi bucks it was 987.00±3.04, 989.00±3.24 and 992.16±2.83 IU/L respectively.

There was a significant difference (P<0.05) between the two breeds of bucks with respect AKP leakage at all freezing stage. Significantly, higher AKP leakage was noted in Osmanabadi than Sirohi bucks.
There was no significant difference (P>0.05) across different dilutors with respect to AKP at pre-freezing (5°C) stage. The leakage of AKP was not affected by different extenders.

Significantly, higher AKP leakage noted was in Osmanabadi than Sirohi bucks. Similar observation was reported by Roy et al. (1960) in cattle and buffalo bulls. They observed that phosphates levels were significantly higher in buffalo semen than the cattle semen.

At pre-freezing stage (5°C) average ACP in TYG, TYD and TYE dilutors for Osmanabadi bucks was 56.66±1.30, 55.72±1.44 and 57.55±1.47 and Sirohi buck it was 54.94±1.04, 56.05±1.79 and 58.27±0.50 IU/l respectively.

There was no significant difference (P>0.05) between the two breeds of bucks as well as across different dilutors with respect to ACP at pre-freezing (5°C)stage.

5.5.9.2 Alkaline phosphates (AKP) and Acid phosphates activity at post-freezing (24 hours) stage

At post-freezing stage (24 hours) the average AKP in TYG, TYD and TYE dilutors for Osmanabadi bucks it was 1026.33±3.19, 1018.00±6.15 and 1034.46±7.70 IU/L and for Sirohi bucks it was 1003.88±4.28, 997.33 ± 3.43 and 1002.55 ± 3.04 IU/L respectively.

There was a significant difference (P<0.05) between two breeds of bucks with respective to AKP in all three dilutors at post-freezing stage (24 hours). Significantly, higher leakage of AKP noted was in Osmanabadi than Sirohi bucks at post-freezing stage.

There was no significant difference across different dilutor with respect to AKP at post freezing (24 hours) stage.

At post-freezing (24 hours) stage the average ACP in TYG, TYD and TYE dilutors for Osmanabadi bucks was 60.27±1.28, 58.11±1.11 and 60.66±0.98 IU/L and for Sirohi bucks it was 62.16±0.82, 61.66±0.68 and 63.22±0.90 IU/l respectively.
There was a significant difference (P<0.05) between two breeds of bucks with respect to ACP at post-freezing (24 hours) stage in TYD dilutor. Significantly higher ACP noted was in Sirohi than Osmanabadi bucks.

There was no significant difference (P>0.05) across different dilutors with respect to ACP at post-freezing (24 hours) stage. Dilutors did not affect ACP at post-freezing (24 hours) stage.

5.5.9.3 Alkaline phosphatase (AKP) and Acid phosphatase activity at post-freezing (72 hours) stage.

At post-freezing stage (72 hours) of AKP in TYG, TYD and TYE diluors for Osmanabadi bucks was 1040.68±5.30, 1028.66±6.24, 1046.73±4.99 IU/L and for Sirohi bucks it was 1014.22±4.34, 1004.44±3.38, 1020.11±4.76 IU/L respectively.

There was a significant difference (P<0.05) between the two breeds of bucks with respect to AKP at post freezing (72 hours) stage. Significantly high ACP was noted in Osmanabadi bucks in TYE dilutor.

There was significant difference (P<0.05) across different dilutors with respect to AKP at post freezing (72 hours) stage.

The results further indicated that in both breeds there was a significant difference between TYD and TYE dilutors with respect to AKP at post freezing (72 hours) stage. AKP leakage was more in TYE than TYD dilutor. No significant AKP leakage noted was in TYG and TYD as well as TYG and TYE dilutors. This indicates that AKP leakage was less in TYD and TYG as compared to TYE dilutor.

At post-freezing (72 hours) stage average ACP in TYG, TYD and TYE dilutors for Osmanabadi bucks was 62.50 ± 1.73, 61.55±1.52 and 64.11 ± 1.32 and Sirohi bucks it was 66.55 ± 0.84, 64.50 ± 1.08 and 69.55 ± 0.72 IU/l respectively.

There was a no significant difference (P>0.05) between the two breeds of bucks respect to ACP at post-freezing (72 hours) stage.

There was no significant difference in both the breeds across different dilutors with respect to ACP at post-freezing (72 hours) stage.
5.5.9.4 Alkaline phosphatase (AKP) and Acid phosphatase (ACP) activity in TYG dilutor

The average Alkaline phosphates (AKP) in TYG dilutor at pre-freezing (5°C), post freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 1001.38 ± 5.19, 1026.33 ± 3.91 and 1040.68 ± 5.30 IU/L and for Sirohi bucks it was 987.00 ± 3.04, 1003.88 ± 4.28, 1014.22 ± 4.34 IU/L respectively.

There was significant difference (P<0.05) between the two breeds of bucks at pre-freezing and post freezing stages. Significantly higher AKP noted was in Osmanabadi than Sirohi bucks.

There was significant difference (P<0.05) at different time interval and temperature with respect to AKP TYG dilutor.

The results further indicated that in Osmanabadi bucks semen there was a significant difference across pre-freezing and 24 hours post-freezing, pre-freezing and 72 hours post freezing as well as between 24 and 72 hours post-freezing stages with respect to AKP in TYG dilutor. This indicates that significant AKP leakage continued in post-freezing stage. Further investigation is necessary to know up to what time the AKP leakage continues at post-freezing stage in Osmanabadi bucks.

In Sirohi bucks semen, there was a significant difference across pre-freezing and 24 hours post-freezing as well as pre-freezing and post freezing (72 hours) stages but there was no significant difference between 24 hours post-freezing and 72 hours post-freezing stage with respect to AKP in TYG dilutor.

The average ACP in TYG dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 56.66±1.30, 60.27±1.28 and 62.50±1.73 IU/L and for Sirohi bucks it was 54.94±1.04, 62.16±0.82, and 66.55±0.84 IU/L respectively.

There was no significant difference (P<0.05) between Osmanabadi and Sirohi bucks in with respect to ACP in TYG dilutor. There was a significant difference (P<0.05) between time interval and temperature with respect to ACP in TYG dilutor.

The results further indicated that in Osmanabadi bucks semen there was no significant difference across different time interval and temperature with respect to ACP in TYG dilutor.

In Sirohi bucks semen there was a significant difference across pre-freezing and post freezing (24 hours) as well as pre freezing and post-freezing (72 hours) with
respect to ACP in TYG dilutor. But there was no significant difference across post freezing 24 and 72 hours with respect to ACP in TYG dilutor.

5.5.9.5 Alkaline phosphatase (AKP) and Acid phosphatase activity in TYD dilutor

The average Alkaline phosphatase (AKP) in TYD dilutor at pre-freezing (5°C), post-freezing (24 hours) and post freezing (72 hours) stages for Osmanabadi bucks was 1003.66±7.71, 1018.00±6.15 and 1028.72±6.24 IU/L and for Sirohi bucks it was 989.00±3.24, 997.33±3.43 and 1004.44±3.38 IU/L respectively.

There was a significant difference (P<0.05) between the two breeds of bucks semen with respect to AKP at pre-freezing and post freezing stage. Higher AKP leakage noted was in Osmanabadi than Sirohi bucks in TYD dilutor.

There was significant difference noted (P<0.05) across different time interval and temperatures with respect to AKP in TYD dilutor.

The results further indicated that in Osmanabadi bucks semen there was a significant difference between pre-freezing and post freezing (24 and 72 hours) stages AKP significantly increased at post freezing (24 and 72 hours) stage than pre-freezing (5°C) stage.

In Sirohi bucks semen there was a significant difference between pre-freezing and post freezing (72 hours) AKP in TYD dilutor. AKP significantly increased at post freezing (72 hours) stage than pre-freezing (5°C) stage.

No significant AKP leakage observed between 24 and 72 hours post-freezing in TYD dilutor in both breeds semen, which indicates that AKP leakage was not significantly increased post-freezing in TYD dilutor.

The average ACP in TYD dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 55.72±1.44, 58.11±1.11 and 61.55±1.52 and for Sirohi bucks it was 56.05±1.79, 61.66±0.68 and 64.50±1.08 IU/L respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to ACP at pre freezine and post freezing (24 and 72 hours) stage in TYD dilutor.

241
There was a significant difference across different time interval and temperatures with respect to ACP in TYD dilutor.

The results further indicated that in both the breeds buck semen there was a significant difference between pre-freezing and 72 hours post-freezing ACP in TYD dilutor. No significant difference noted between post freezing 24 and 72 hours ACP in both breeds semen in TYD dilutor, which indicates that in TYD dilutor ACP leakage was not significant after freezing.

5.5.9.6 Alkaline phosphatase (AKP) and Acid phosphatase activity in TYE dilutor

The Alkaline phosphates (AKP) in TYE dilutors at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 1008.76±6.02, 1034.46±7.70, 1046.73±4.99 IU/L and for Sirohi bucks it was 992.16±2.83, 1002.55±3.04 and 1020.11±4.76 IU/L respectively.

There was a significant difference (P<0.05) between the two breeds of bucks at different time interval and temperatures. AKP was significantly higher in Osmanabadi than Sirohi bucks at pre-freezing and post freezing (24 and 72 hours) stage in TYE dilutor.

There was a significant difference (P<0.05) between different time interval and temperatures with respect to AKP in TYE dilutor.

The results further indicated that in Osmanabadi bucks semen there was significant difference across pre-freezing and post freezing (24 hours) as well as pre-freezing and post freezing (72 hours) stage with respect to AKP in TYE dilutor. Significantly, higher AKP noted was at post freezing 24 and 72 hours than pre-freezing stage. No significant difference was noted between post freezing (24 hours) and post freezing (72 hours) stage, it indicates that AKP was not significantly increased after 24 hours of freezing in Osmanabadi bucks indicating no significant increase in sperm damage.

In Sirohi bucks semen, there was significant difference between pre-freezing and post freezing (72 hours) as well as post freezing (24 hours) and post freezing (72 hours) stage with respect AKP in TYE dilutor. As there was significant increase in AKP
post freezing (72 hours) than post freezing (24 hours) indicates that sperm damage was significantly increasing in Sirohi bucks post freezing (72 hours) in TYE dilutor.

The average Acid phosphatase (ACP) in TYE dilutors at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 57.55±1.47, 60.66±0.98, 64.11±1.32 IU/L and for Sirohi bucks it was 58.27±0.50, 63.22± 0.90 and 69.55 ± 0.72 IU/L respectively.

There was no significant difference (P>0.05) between the two breeds of bucks with respect to ACP in TYE dilutor.

There was no significant difference (P>0.05) across different time interval and temperatures with respect to ACP in TYE dilutor.

5.5.10 Lactate dehydrogenase (LDH) activity

The Lactate dehydrogenase (LDH) at different freezing stages in different dilutors of Osmanabadi and Sirohi bucks semen was recorded as below.

5.5.10.1 Lactate dehydrogenase (LDH) at pre-freezing (5°C) stage

At pre-freezing stage (5°C) average LDH in TYG, TYD and TYE dilutors for Osmanabadi bucks was 308.26 ± 5.02, 309.25±4.34 and 315.56 ± 5.51 IU/L and for Sirohi bucks it was 297.27 ± 8.47, 298.05 ± 8.92 and 302.61 ± 8.42 IU/L respectively.

There was no significance difference (P>0.05) between the two breeds of bucks with respect to LDH at pre-freezing (5°C).

There was no significance difference (P>0.05) across different dilutors with respect to LDH at pre-freezing (5°C) stage. This indicates that LDH was not affected by dilutors in both the breeds.
5.5.10.2 Lactate dehydrogenase (LDH) at post-freezing (24 hours) stage

At post-freezing (24 hours) stage average LDH in TYG, TYD and TYE dilutors for Osmanabadi bucks was 320.83±6.03, 319.55±4.49 and 321.06±5.76 IU/L and for Sirohi bucks it was 306.66±8.24, 310.49±9.75 and 308.7±9.71 IU/L respectively.

There was no significance difference (P>0.05) between the two breeds of bucks as well as across different dilutors with respect to LDH at post-freezing (24 hours) stage.

5.5.10.3 Lactate dehydrogenase (LDH) at post-freezing (72 hours) stage

At post-freezing (72 hours) stage average LDH in TYG, TYD and TYE dilutors for Osmanabadi bucks was 325.88±5.31, 324.2±4.83 and 331.50±5.62 IU/L and for Sirohi bucks it was 320.55±8.33, 316.83±11.54 and 327.27±11.49 IU/L respectively.

There was no significant difference between the two breeds of bucks as well as across different dilutors with respect to LDH at post freezing (72 hours) stage.

5.5.10.4 Lactate dehydrogenase (LDH) in TYG dilutor

The average Lactate dehydrogenase (LDH) in TYG dilutor at pre-freezing (5°C), post freezing(24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 56.33±1.30, 60.33±1.28 and 62.50±1.73 IU/L and for Sirohi bucks it was 54.94±1.04, 62.17±0.82 and 66.56±0.84 IU/L respectively.

There was no significant difference between the two breeds of bucks as well as there was no significance difference across different time interval and temperatures respect to LDH in TYG dilutor.
5.5.10.5 Lactate dehydrogenase (LDH) in TYD dilutor

The average LDH in TYD dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 309.25±4.34, 318.67±4.49 and 324.19±4.83 IU/L and for Sirohi bucks it was 298.05±8.92, 310.50±9.75, 316.83±11.54 IU/L respectively.

There was no significant difference (P>0.05) between the two breeds of bucks as well as no significant difference (P>0.05) across different time interval and temperatures with respect to LDH in TYD diluter. LDH was not affected pre and post-freezing stage in TYD dilutor in semen of Osmanabadi and Sirohi bucks.

5.5.10.6 Lactate dehydrogenase (LDH) in TYE dilutor

The average Lactate dehydrogenase (LDH) in TYE dilutor at pre-freezing (5°C), post-freezing (24 hours) and post-freezing (72 hours) stages for Osmanabadi bucks was 315.55±5.51, 321.17±5.76 and 331.50±5.62 IU/L and for Sirohi bucks it was 302.61±8.42, 308.72±9.71, 327.28±11.49 IU/L respectively.

There was no significance difference (P>0.05) between the two breeds of bucks as well as there was no significance difference (P>0.05) across different time interval and temperatures with respect to LDH in TYE dilutors except in Sirohi bucks there was significant leakage of LDH from pre freezing to post freezing (72 hours) stage.
5.6. Study of conception rate of frozen semen

5.6.1 Conception rate of Osmanabadi buck frozen semen

The conception rate of Osmanabadi buck semen frozen in straws in TYG, TYD and TYE dilutor in oestrous synchronized does was 33.33, 38.88 and 19.44 percent respectively. The overall conception rate for Osmanabadi buck frozen semen was 30.55 percent.

Higher conception rate was observed for semen frozen in TYD dilutor as compared with TYE and TYG dilutor. Higher conception rate was also observed for TYG as compared to TYE dilutor. It indicates that DMSO was best for freezing Osmanabadi bucks semen in straws compared to glycerol and ethylene glycol.

5.6.2 Conception rate of Sirohi buck frozen semen

The conception rate of Sirohi buck semen frozen in straws in TYG, TYD and TYE dilutor in oestrous synchronized does was 30.55, 36.11 and 27.77 percent respectively. The overall conception rate for Sirohi buck frozen semen was 31.48 percent.

Higher conception rate was observed for semen frozen in TYD dilutor as compared with TYE and TYG dilutor. Higher conception rate was also observed for TYG as compared to TYE dilutor. It indicates that DMSO was best for freezing Sirohi bucks semen compared to glycerol and ethylene glycol.

5.6.3 Overall conception rate of Osmanabadi and Sirohi buck frozen semen in different dilutors

The average conception rate of semen frozen in TYG, TYD and TYE dilutors for Osmanabadi bucks was 33.33, 36.11 and 19.44 percent and for Sirohi bucks it was 30.55, 38.88 and 27.77 percent respectively.
The overall conception rate of semen frozen in TYG, TYD and TYE dilutors was 31.94, 37.49 and 23.60 % respectively. Higher conception rate was observed for TYD dilutor than TYG and TYE dilutors. TYG dilutor also showed higher conception rate than TYE dilutor. It indicates that TYD was the best dilutor followed by TYG for freezing Osmanabadi and Sirohi buck semen. This also indicates that fertility of spermatozoa was better preserved when DMSO was used as cryoprotectant than glycerol or ethylene glycol.

5.7 Evaluation of Boer and Damascus bucks pelleted frozen semen received from research organization

5.7.1 Evaluation of semen quality of pellets of Boer and Damascus breed of bucks.

In Boer and Damascus bucks frozen semen average microscopic semen attributes such as post thaw sperm motility percentage was 65.00±1.82 and 65.00±2.24, live sperm percentage was 74.67±1.14 and 77.50±2.25, abnormal sperm percentage was 19.00±0.97 and 20.17±0.83 respectively.

In Boer and Damascus bucks frozen semen average sperm function test attributes such as hypo osmotic swollen sperm percentage was 64.33±1.48 and 64.67±1.52, acrosome intact sperm percentage was 85.67±1.71 and 85.33±2.26, DNA damaged sperm percentage by Acridine orange test was 8.83±0.60 and 11.50±0.99 and DNA damaged sperm percentage detected by Comet assay was 0.83±0.40 and 1.00±0.37 respectively.

In Boer and Damascus bucks frozen semen average GOT was 80.33±0.95 and 85.67±0.92 IU/L, GPT was 19.33±1.16 and 18.33±0.95 IU/L, AKP was 889.17±8.44 and 955.17±14.22 IU/L, ACP was 46.00±2.00 and 45.50±1.26 IU/L and LDH was 301.17±4.18 and 313.00±4.76 IU/L respectively.
5.7.2 Conception rate of Boer and Damascus bucks pelleted frozen semen

The conception rate of Boer and Damascus bucks pelleted frozen semen was 25 and 16.66 percent respectively.

The Boer buck frozen semen had showed higher conception rate as compared to Damascus buck frozen semen.
CONCLUSIONS

From the present research work following conclusions can be drawn.

1) The macroscopic, microscopic, sperm function test and enzyme activity in neat semen of Osmanabadi and Sirohi bucks indicated that the quality of neat semen was very good.

2) The microscopic, sperm function test and enzyme activity in the diluted semen at 5°C (Pre-freeze) with different dilutors like TYG, TYD and TYE indicated that there was slight reduction in the quality of semen as compared to neat semen.

3) There was no significant difference observed between the breeds but significant difference was observed between the dilutors at all three stages i.e. pre-freeze, post-freeze 24 and 72 hours stage. TYD dilutor was found to be superior to TYE and TYG dilutors at all the three stages.

4) When the post-thaw quality of semen was analyzed, it was noted that Osmanabadi buck semen in all dilutors at different time intervals was better than that of Sirohi buck semen, particularly for post thaw motility percentage and plasma membrane integrity percentage.

5) In all dilutors post thaw quality of semen was gradually reduced i.e. reduction in post thaw motility percentage and live sperm percentage, increase in sperm abnormality percentage, DNA damaged sperm percentage and enzyme leakage. This reduction in quality was within the permissible limit. However, the post-thaw plasma membrane integrity and acrosomal intactness was found to be fairly constant.

6) The conception rate of semen frozen in TYE dilutor was higher for Sirohi than Osmanabadi bucks, but conception rate of semen frozen in TYD and TYG dilutors was higher in Osmanabadi bucks than Sirohi bucks. This indicates that dilutor plays an important role in semen preservation.

Thus from the results obtained in the present research work, it can be concluded that the quality of diluted semen declines gradually during freezing of...
semen from pre-freeze (5°C) to post thaw (72 hours) stage. However, this decline is gradual and within the normal permissible limits. Therefore, the quality of semen at 72 hours of freezing was good and the frozen semen thus prepared can be undoubtedly used for Artificial Insemination. Amongst the breeds better conception rate with AI of Sirohi bucks frozen semen and amongst the dilutors better conception rate with TYD dilutor were the important findings of this project.
6. PROPOSED AREA FOR FUTURE RESEARCH WORK

**Multiple Ovulation & Embryo Transfer (MOET)** MOET is one of the best technology for genetic improvement through selection of superior does & bucks.

**In-vitro fertilization (IVF)**, Chimeras, Cloning by nuclear transplantation & Transgenic animals, these are the few new technologies, which are available. These technologies can be exploited by using preserved semen.

**Production of Transgenic Goats by Sperm-mediated Exogenous DNA.** DNA transfer method for production of transgenic goats using elite germ plasm sperm to integrate exogenous DNA through artificial insemination. AI can be carried out and the protocols for sperm-mediated gene transfer (SMGT) in the goats can be optimized for enhancing productivity.

**Flow cytometric sex-sorting and cryopreservation of elite bucks spermatozoa.**

**Impacts of oxidative stress and antioxidants on semen functions.**
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256


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# Thesis Abstract

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<tr>
<th>a) Title of thesis (in capital letters)</th>
<th>DEEP FREEZING OF OSMANABADI AND SIROHI BUCK SEMEN WITH SPECIAL REFERENCE TO SPERM MEMBRANE INTEGRITY, DNA DAMAGE AND FERTILIZING ABILITY</th>
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<td>b) Full name of student</td>
<td>Dagli Nilesh Rameshchandra</td>
</tr>
<tr>
<td>c) Name and address of Major Advisor</td>
<td>Dr. S. A. Bakshi, Professor of Gynaecology, Bombay Veterinary College Parel, Mumbai-12.</td>
</tr>
<tr>
<td>d) Degree to be awarded</td>
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<td>f) Major subject</td>
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<td>j) Signature Name and address of forwarding authority (HOD/SH)</td>
<td>Dr. S. A. Bakshi, Professor of Gynaecology, Bombay Veterinary College</td>
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Associate Dean  
Bombay Veterinary College
ABSTRACT

The present research work was done on semen 36 semen samples of 12 (6 Osmanabadi and 6 Sirohi) bucks for various macroscopic, microscopic, sperm function tests and enzyme activity on neat semen and on semen diluted with TYG (Tris egg yolk glycerol), TYD (Tris egg yolk di-methyl sulphoxide) and TYE (Tris egg yolk ethylene glycol) diluters respectively, at three stages of pre-freezing (5°C), post freezing (24 hours) and post freezing (72 hours) respectively. The macroscopic, microscopic, sperm function tests and enzyme activity in neat semen of Osmanabadi and Sirohi bucks indicated that the quality of neat semen was very good and within normal prescribed limits. Similarly the microscopic, sperm function tests and enzyme activity in the semen of Osmanabadi and Sirohi bucks diluted with different diluters like TYG, TYD and TYE respectively indicated that there was slight reduction in the quality of semen as compared to neat semen. There was no significant difference between the breeds but significant difference was observed between the diluters at all three stages i.e. pre-freeze, post-freeze 24 and 72 hours. TYD diluter was found to be superior than TYG and TYE at all the three stages i.e. pre-freezing (5°C), post freezing 24 and 72 respectively. When the post-thaw quality of semen was analyzed, it was noted that Osmanabadi buck semen in all diluters at different time intervals was better than that of Sirohi buck semen, particularly for post thaw motility percentage and plasma membrane integrity percentage. In all diluters post thaw quality of semen was gradually reduced i.e. reduction in post thaw motility percentage and live sperm percentage, increase in sperm abnormality percentage, DNA damage sperm percentage and increase in enzyme leakage. This reduction in quality was within the prescribed limit. However, the post thaw plasma membrane integrity and acosomal intactness was found to be fairly constant. There were indications of
increased leakage of enzymes into the plasma from pre freezing (5°C) to post thaw (24 and 72 hours) stage.

Total 216 oestrous synchronized goat were inseminated with frozen semen of Osmanabadi and Sirohi bucks. Conception rate of semen frozen in TYE diluter was higher for Sirohi than Osmanabadi bucks, but conception rate of semen frozen in TYD and TYG diluter was higher in Osmanabadi than Sirohi bucks. The overall conception rate of frozen semen was higher in Sirohi than Osmanabadi bucks. This indicates that diluter plays an important role in semen preservation.

Thus from the results obtained from the present research work, it can be concluded that the quality of diluted semen declines gradually during freezing of semen from pre-freeze (5°C) to post thaw (72 hours) stage. However, this decline is gradual and within the normal permissible limits. Therefore, the quality of semen at 72 hours of freezing was good and the frozen semen thus prepared can be undoubtedly used for Artificial Insemination. Amongst the breeds better conception rate with AI of Sirohi bucks frozen semen and amongst the diluter better conception rate with TYD diluter were the important findings of this project.
प्रबंध सारांश

१. प्रबंधाचे नाव :
आणि सिरोही ओमानाबादी भोकडाच्या वीणाचे गोठवणूक, प्रामुख्याने शुकापुंच्या आवरणाची तपासणी, डॉ. एन. ए. ला होणाच्या नुकसानीची तपासणी आणि श्यामेची तपासणी

प्रजनन

२. विद्याध्यापक नाव :
श्री. डगली निलेश रमेशचंद्र

३. मार्गदर्शिकाचे नाव आणि पत्ता :
डॉ. श्रीगांव अ. बक्की, प्राध्यापक पशु प्रजनन शास्त्र, मुंबई पशुवंडक महाविद्यालय, परवंड, मुंबई - १२

४. पदवी :
पद्ध्युलतर/आचार्य पदवी

५. पदवी प्रदान करण्याचे वर्ष :
२०१२

६. मुख्य विषय :
शकु प्रजनन शास्त्र

७. प्रबंधाची एकूण पाने :
२२७

८. सारांशाचे सहित एकूण शब्द :
४०९

९. विद्याध्यापक सही :

१०. विभाग प्रमुखाचे नाव, सही आणि पत्ता :
डॉ. श्रीगांव अ. बक्की, प्राध्यापक पशु प्रजनन शास्त्र, मुंबई पशुवंडक महाविद्यालय, परवंड, मुंबई - १२
सारांश

उस्मानाबादी आणि सिरोही बोकडंगाच्या वीर्यांचा गोठवणूक, प्रामुख्याने शुक्राणुंचा आवरण तपासणी, डॉ. एन. ए. ला नोटाच्या नुकसानीची तपासणी आणि प्रजनन शक्तीची तपासणी

या संरक्षण प्रकल्पात एकूण 36 बोकडंगाच्या वीर्यांमध्ये (६ उस्मानाबाद व ६ सिरोही) संरक्षण करण्यात आलेले. या बोकडंगांच्या वीर्यांना कृतीय योगी वापरले गेलेले संकल्पना करण्यात आलेले. या वीर्यांच्या विविध चार्च्यांना ध्येय आल्या. सुखमदर्श वापरले, सुखमदर्शक न वापरला, वीर्यांतील वीरकंपंच चार्च्या (जीओटी/जीपीटी/ए/सी.पी. /ए/सी.पी. /एक.डी.एच.) शुक्राणुंचा आवरणाची तपासणी आणि डॉ. एन. ए. ला नोटाच्या नुकसानीची तपासणी. त्या नंतर उकृठ विविध विषयांमध्ये ५° से. तापमानाच्या विविध दाबाने वापरले गोठवणूक करण्यात आलेली. ५° से. ला साठवणूक केलेल्या वीर्यांच्या विविध चार्च्यांना ध्येय आल्या (सुखमदर्श चंद्र वापरले शुक्राणुंच्या तपासणी, शुक्राणुंच्या आवरणाच्या चार्च्या, डॉ. एन. ए. ला नोटाच्या नुकसानीची तपासणी, वीर्यांतील वीरकंपंच चार्च्या)

व वीर्यांतील प्रत ठरवण्यात आली. ५° से. नंतर दाबानून (टिस, आंडावाचा बल्क गोलीसारळ TYG/टिस आंडावाचा बल्क डि.एम.एस.ओ. TYD /टिस आंडावाचा बल्क इथियोपियन फार्मूल TYE) वीर्यांतील - १८६° से. तापमानाच्या साठवणूक करण्यात आली. -१८६° से. ला साठवणूक केलेल्या वीर्यांतील २४ आणि ७२ तासाचा वरील निर्देशीत चार्च्या वापरले प्रत्येक ३ तासाच्यात ठरवण्यात आली.

वीर्यांच्या विविध चार्च्यांमध्ये नियमपूर्वक खालील गोष्टी लक्षण आल्या:

१. उस्मानाबादी आणि सिरोही बोकडंगांच्या वीर्य हे उकृठ प्रकाराचे वीर्य होते.

२. ५° से. तापमानाच्या जेता उस्मानाबादी आणि सिरोही बोकडंगांचे वीर्य विविध दाबाने

(TYG/TYD/TYE) साठविले जाते त्यामध्ये व्याही प्रत धोडीसो कमी होते.
३. -१९६९ से. तापमानाचा विविध द्राक्षण (TYG/TYD/TYE) गोठिकलेख्या विनियमाध्ये प्रतारीता मध्ये बराच फरक जाणविलेला. प्रतारीता कमी होतेस. त्यामध्ये TYD द्राक्षणातील वीर्यांची प्रतिही इतर दोन द्राक्षणातील (TYG/TYE) वीर्यांच्या प्रतीपेश्व चांगली असते.

४. विविध द्राक्षण गोठिकलेख्या वीर्यांच्या (-१९६५ से.) सिरोही बोकडांचे व उस्मानाबादी बोकडांच्या वीर्यांची (शुक्राणुवे हालात % आणि शुक्राणुवे आवरणाची संशोधनता %) प्रतवारी चांगली असते.

५. गोठिकलेख्या वीर्यांच्या प्रत ही न गोठिकलेख्या अथवा ५° से. ला साठिकलेख्या वीर्यांच्या प्रतीपेश्व कमी असते परंतु हया प्रतीचे वीर्य कृत्रिम येतन कण्यासाठी योग्य असते.

६. २१६ रोज्यांमध्ये गोठिकलेख्या वीर्यांचा कृत्रिम रेताचा वापर करून झालेल्या गर्भधारणे वरन असा निष्क्रिय निघतो की,

अ) सिरोही बोकडांसह TYD द्राक्षणातील वीर्यांमध्ये होणार्या गर्भधारणाचे प्रमाण जास्त असते.

ब) उस्मानाबादी बोकडांसह TYD आणि TYG द्राक्षणातील वीर्यांमध्ये होणार्या गर्भधारणाचे प्रमाण जास्त असते.

सर्वसाधारणपणे सिरोही बोकडांच्या गोठिकलेख्या वीर्यांना होणारे गर्भधारणाचे प्रमाण उस्मानाबादी बोकडांच्या गोठिकलेख्या वीर्यांने होणार्या प्रमाणपेक्षा जास्त असते.

सदृश संशोधनातून असा निष्क्रिय काहीत येतो की, उस्मानाबादी आणि सिरोही बोकडांंचे वीर्य गोठिकलेख्या त्याच्या प्रतवारी कमी होते परंतु गोठिकलेख्या नंतरची प्रतवारी कृत्रिम रेताचा वापरणाचा जोगी चांगली असते. सिरोही बोकडांसह गोठिकलेख्या वीर्यांचा वापर केलंजवा कृत्रिम रेतामध्ये गर्भधारणाच्या महत्वाच्या शाहसाखांच्या प्रमाण हे उस्मानाबादी बोकडांच्या गोठिकलेख्या वीर्यांच्या कृत्रिम रेतामध्ये गर्भधारणाच्या महत्त्वाच्या
રાખણાતંશ શેષયાંચા પ્રમાણાંકણ જાત અસાર. સાથે દ્રાઘણામ્બયે વિવિધ પ્રત્યારીત TYD દ્રાગણાચી
પ્રતિ શિક્ષણ ચંગલી અસાર.
VITA

The author Mr. Nilesh Rameshchandra Dagli was born on 12th July, 1973 at Murbad, District-Thane, Maharashtra State. He passed S.S.C examination from Model High School, Taluka - Dahanu Road, District –Thane in 1988 with distinction. Then he passed H.S.C examination in 1990 from Godrej Technical Institute, Bordi, Dist-Thane with first class. He opted Veterinary Science for further studies and graduated (B.V.Sc and A.H) with first class from Bombay Veterinary College in the year 1995. He has done masters (M.V.Sc) in the discipline of Animal Reproduction from Bombay Veterinary College in the year 1997 and received a gold medal for the same. He has also qualified the NET (National Eligibility Test) examination. He is working as academic faculty member at Bombay Veterinary College since September 1997. He is well acquainted with computers and also secured First class in Advanced Diploma in Computer Software System Analysis & Application (ADCSSAA), which is recognized by Maharashtra State Board of Technical Education. He has published research and extension article in National Journals. He is CO-Principal Investigator for two National research projects. He is also acting as Coordinator for training programs conducted at Instructional Livestock Farm Complex, Bombay Veterinary College.