SEMINAL CHARACTERISTICS AND FREEZABILITY OF MURRAH BULL SEMEN DURING SPRING AND SUMMER SEASONS

Thesis

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VITA

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CERTIFICATE – I

This is to certify that the thesis entitled **"SEMINAL CHARACTERISTICS AND FREEZABILITY OF MURRAH BULL SEMEN DURING SPRING AND SUMMER SEASONS"** submitted in partial fulfillment of the requirements for the degree of **"Master of Veterinary Science"** of the Indira Gandhi Krishi Vishwavidyalaya, Raipur, is a record of bonafide research work carried out by Mr. **Subodh Kumar** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma (certificate awarded etc.) or has been published / published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by him.

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CERTIFICATE – II

This is to certify that the thesis entitled **"SEMINAL CHARACTERISTICS AND FREEZABILITY OF MURRAH BULL SEMEN DURING SPRING AND SUMMER SEASONS"** submitted by **Subodh Kumar** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur, in partial fulfillment of the requirements for the degree of **Master of Veterinary Science** in the Department Of Animal Reproduction, Gynaecology And Obstetrics, College of Veterinary Science and Animal Husbandry, Anjora, Durg has been approved by the Examination Committee after oral examination.

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LIST OF ABBREVIATIONS/SYMBOLS

A.I.	:	Artificial insemination
A.V.	:	Artificial vagina
µg.	:	Microgram
cm.	:	Centimeter
CMPT.	:	Cervical mucus penetration test
et al.	:	Et alli
Fig.	:	Figure
Gm.	:	Gram
HOST.	:	Hypo-osmotic swelling test
Hr (s)	:	Hour
Kg.	:	Kilogram
i.e.	:	That is to say
I.U.	:	International Unit
L.	:	Liter
lb.	:	Pound
Min.	:	Minute (s)
ml.	:	Millilitre
mm.	:	Millimeter
mmol/kg	:	millimol per kg
No.	:	Number
PTM.	:	Post-thaw motility
Sec.	:	Second
S.E.	:	Standard Error
viz.	:	Namely
Symbol	**:**	**Full form**
%	:	Percentage
^{o} C	:	Degree Celsius
@	:	At the rate of
<	:	Less than
>	:	More than

CHAPTER – I

INTRODUCTION

Livestock sector is an integral component of Indian agriculture, contributing about 31% of total agrarian economy of the country. India ranks first in the milk production in the world with an estimated production of about 100.9 million tones (GOI, 2006-07). Buffalo population in India is half to that of cattle and accounts about 55% of total milk production of the country (GOI, 2006-07). Livestock sector plays a multifaceted role in socio-economic development of households. It contributes about 6 percent to the Gross Domestic Product. Over the last two decades, livestock sector has grown at an annual rate of 5.6 percent, which is higher than 3.3 percent growth in agricultural sector. This suggests that livestock is likely to emerge as an engine of agricultural growth in the coming decades.

Majority of the buffaloes in our country are non descript with an average milk yield of only 541 kg per lactation which is comparatively less than the average milk yield of 1597 kg recorded for Murrah, one of the recognized buffalo breeds of our country (Pundir and Sahai, 1997). The frozen semen of Murrah bull is extensively used for augmenting milk production among the local buffaloes in our country (Banerjee, 1991) for genetic upgradation. The objective is to improve milk production of our buffalo stock rapidly by exploiting the superior germplasm. The accurate evaluation of semen immediately after collection and freezing is necessary as it's lower fertility potential brings the artificial insemination to a disrepute. The semen production traits viz. volume, initial

motility, sperm concentration, prefreeze and post-thaw motility of breeding bulls have considerable importance to decide for utilizing their semen for artificial insemination. The post thaw motility otherwise termed as the freezability of semen, is the ultimate trait, which qualifies the semen for artificial breeding. Besides producing large quantity of milk, buffalo also contributes towards meat production and animal drought power.

Fertility is a measure of reproductive success. To assess reproductive success most logical & accurate method is to assess pregnancy rate and non return rates. For successful reproduction, male animal should produce good quality semen and perform sexual act in a normal way. The evaluation of bulls their aptitude to reproduce and for the fertilizing ability of their semen requires assessment of their reaction time during semen collection. Reaction time is highly correlated to semen production. Spermatozoal motility, live sperm percent, abnormal sperm percent, acrosomal abnormities, cervical mucus penetration test & hypo-osmotic swelling test are some of the attributes which provides a reasonably reliable estimate of the semen samples quality as well as freezability.

Successful cryopreservation of semen is the backbone of frozen semen technology, with the development of frozen semen technology, the demand for outstanding sires has increased considerably. This has led the requirement to develop the methods for predicting potential sperm production and particularly, for identifying bulls with high sperm freezability at an early age. Cryo-injury and handling during storage results in large number of infertile sperms due to physical, osmotic and chemical damages. Several investigators have extensively studied the testicular growth and related semen characteristics in the post pubertal dairy bulls (Foote *et al*., 1977; Hahn *et al*., 1969). Correlation between testicular biometry

with semen production may be useful for breeding soundness evaluation of Murrah buffalo bulls. Determination of scrotal circumference is an essential aspect of breeding soundness examination and has great value as an indicator of puberty, total sperm production, semen quality, pathological conditions of the testes, as well as the sub fertility or infertility of a bull (Lunstra *et al*., 1978; Ahmad *et al*., 1989 and Ott, 1991). More importantly, scrotal circumference is highly inheritable (Ott, 1991) and is related to age of puberty in daughter and half-sib heifers and their subsequent fertility and productivity (Brinks *et al*., 1978). Both body mass and scrotal circumference are reported to be affected by seasons (Ahmad *et al*., 1989; Younis, 1996). Chenoweth (1980) has shown that a highly significant positive correlation of 0.58 exists between the scrotal circumference and the fertility rate of bulls and could be used for predicting fertility of young bulls and also as one of the important criteria of evaluating breeding values of bulls. Testicular volume also gives high correlation (+ve) with the fertility rate of bulls.

The size of testis particularly scrotal circumference is an important parameter in breeding bull by virtue of its high correlation with sperm output in bulls (Almquist and Amann 1961), semen quality (Hahn *et al*., 1969), as well as fertility (McCosker *et al*., 1989).

Semen quality of animals is traditionally measured by evaluating certain physical parameters like sperm count, motility and morphology for the assessment of male fertility. However, these visibly measurable sperm characteristics have low correlations and low predictive values to male fertility (Amann, 1989). The fertility of an ejaculate of semen is the ultimate in it's evaluation. This can be accomplished only by inseminating females and waiting long enough to determine

whether or not they return to estrus or until a pregnancy diagnosis can be made. The ability to evaluate sperm characteristics *in-vitro* is essential to establish a correlation between the importance of any characteristics and overall fertilizing ability. Accordingly, attempts are being made to develop tests capable of predicting fertility with greater accuracy. One such test is termed hypo-osmotic swelling test (HOST) which was initially developed to evaluate biochemical activity of the physically intact human sperm membrane (Jeyendran *et al*., 1984). This test is dependent upon the positive correlation between coiling of flagellum in response to lowered osmotic pressure and high fertilizing ability of the semen (Van der Ven *et al*., 1986). This test has been developed for diverse mammalian species such as cattle (Correa and Zavos, 1994; Revell and Mrode, 1994) horses (Caiza de la Cueva, 1997) and pigs (Gadea *et al*., 1998). Other test which is capable of predicting fertility with greater accuracy is cervical mucus penetration test which is initially developed by Kremer in 1965. Hypo–osmotic swelling test and cervical mucus penetration test gives greater accuracy to predict viability and functional integrity of spermatozoa.

Freezing affect not only post thaw motility but may have functional and structural changes on sperm which might affect the fertility of semen. So it is important to study all parameters after freezing and to study the effect of duration of incubation period on post thaw seminal characteristics.

Routine semen evaluation methods (i.e. total number of spermatozoa, progressive motility and morphology) have limited accuracy in predicting the fertility of a semen samples (Bishop *et al*., 1954; Linford *et al*., 1976; Soderquist *et al*., 1991). Murrah bulls is highly sensitive to agroclimatic changes particularly temperature, Although buffalo bulls breed the year round, conflicting reports have

been published about semen quality and volume at different ages and during various seasons of the year. Better semen quantity of buffalo bull has been reported during the hot and humid summer than in colder months (Wierzbowski and Tahir, 1980; Singh *et al*., 1992) but contrary observations have also been published (Li *et al*., 1988). Similarly, significantly ($P<0.05$) inferior (Erb *et al*., 1942; Bhattacharya 1968; Bhosrekar, 1980; Rao, 1984) and superior semen quality has been reported during summer (Singh *et al*., 1992). Saeed (1988) reported the best quality semen at 3-4 years of age. Therefore it is important to study the season effect on different seminal characteristics.

The level of liquid nitrogen in container during storage and transportation is also reported to have significant influence on sperm survival, so that it is quite interesting to see the effect of level of liquid nitrogen on post thaw seminal characteristics.

Therefore the present research work is designed to study:

1. The correlation of testicular biometry and reaction time with semen production.
2. The physio-morphological characteristics and freezability of Murrah bull semen during spring and summer seasons.
3. The effect of duration of post thaw incubation period on post thaw motility, hypo-osmotic swelling test & cervical mucus penetration test of spermatozoa.
4. Effect of level of liquid nitrogen in container on post thaw seminal characteristics.

CHAPTER – II

REVIEW OF LITERATURE

Artificial insemination is arguably the most important tool contributing to the advancement of modern dairy farming. As artificial insemination (A.I.) is advantageous over natural service in many aspects.

Semen preservation at a large scale is desirable for A.I. programmes. However, the success of the A.I. programme is dependent on the successful cryopreservation of spermatozoa under *in vitro* storage conditions. Many attempts in the past have been made towards judging the semen quality and improving freezability of bull semen (Roy *et al*., 1975; Biswas *et al*., 1976 and Prasad, 1997). In this regard, relevant literature dealing with various aspect of semen cryopreservation, damage during freezing with reference to Murrah bull semen is reviewed.

The success of semen storage depends on numerous factors, which may be peculiar to each species and are optimized according to type of semen to be preserved. In the last few years, considerable attention has been paid for improving the freezability and post-thaw recovery rate of spermatozoa used for artificial insemination of Murrah bulls.

2.1.1 Scrotal Circumference and Scrotal Volume

Willett and Ohms (1955) recorded a correlation of 0.32 between scrotal circumference and spermatozoon production over a period of 5 months. Subsequently, Willett and Ohms (1957) obtained a correlation of 0.91 ($p<0.01$) between testicular circumference and average sperm production per exhaustion for

16 exhaustion series with young bulls and a correlation of -0.34 ($p<0.05$) with aged bulls. Hooker studied the endocrine and exocrine functions (1937) and post-natal increase in size of bull testes (1944) and opined that the increase in testes size was largely due to tubular changes and a steady increase in Leydig's cells. Joshi *et al*. (1967) reported that scrotal circumference of adult none descript buffalo bull was 25.6 centimeter.

Thibier (1972) found no relation between size of testis and sperm output in young French-Friesian bulls when frequently ejaculated, however, in bulls subjected to frequent ejaculation after adequate sexual preparation the length of testis showed a significant correlation with sperm concentration as well as total sperm count. Coulter *et al*. (1975) reported a high rate of testicular growth in young Holstein Friesian bulls, limited growth in mature bulls and a slight decrease in old bulls with large difference in testis size among bulls of same age. Elmore *et al*. (1976) reported that breed has no significant effect on scrotal circumference, and suggested that a scrotal circumference of more than 32 cm was necessary for a bull to be classed as a potentially satisfactory breeder.

The correlation between scrotal circumference with semen quality was reported significantly positive in cattle by Morrow (1980). However Bhosrekar *et al*. (1980) contradicted the same. The oblong type of scrotum is most common (69.77%) variety in Murrah bulls followed by square (20.93%) and overlapping (9.30%) type. However, the semen characteristics did not vary significantly among buffalo bulls with different types of scroti (Bedi, 1980; Somasekharam and Ramamohan Rao, 1986). Bedi. (1980) also reported that maximum sperm concentration was attained at 45 to 54 months of age and decreased thereafter. Foote (1984) reviewed the subject of general evaluation of male reproductive

capacity and concluded that size of testis meets all the requirements. The total sperm per ejaculate increased progressively till the age of 7 years and then declined in Murrah bulls, whereas Narsimharao *et al*. (1991) observed that semen volume and total sperms per ejaculate increased with age of the bulls beyond 7 years, and the bulls above 10 years had the highest mean value for the same parameters.

Narsimharao and Venkataramaiah (1993) reported that linear testicular length and scrotal circumference increased significantly with increasing age of Murrah bulls in all age groups. Suryaprakasham *et al*. (1993) observed that testicular dimensions in Murrah bulls increased with age found that bulls with higher trans scrotal circumference produced better semen quality and good sperm morphology in Surti buffalo bulls. Thus, trans scrotal circumference could be used to know the sperm production potential and for selecting superior bulls with enormous economic importance. Factors like breed, age, season and nutrition can affect the testicular size and firmness (Shukla *et al*., 1992; Veerapandiyan *et al*., 1992).

Narsimharao and Venkataramaiah (1993) reported scrotal circumference of Murrah bulls at the age of 2.1 to 2.5 years is 26.5 cm. and between 2.6 to 3.0 years is 28.1 cm.

Narasimharao *et al*. (1993) reported scrotal circumference in the age group 30.1 to 36 month and 36.1 to 42 month of age is 26.7 and 30.0 cm respectively.

Nema and Kodagali (1994) reported mean trans-scrotal circumference was 36.25 cm. in 19 to 46 month age in Surti buffalo. Body weight, sperm

concentration, ejaculate volume and live sperm percent were positively associated with higher trans-scrotal circumference.

Rana and Bilaspuri (1999) reported that scrotal circumference in Murrah bulls above 6 years was 32.5±0.80 centimeter and scrotal circumference increases significantly ($p<0.05$) from 1 to 24 months.

Pant *et al*. (2003) reported that scrotal circumference (30.4 cm) in Murrah bulls age between 49 to 60 month and this was highly correlated with sperm concentration and ejaculate volume. Bulls aged above 5 years had scrotal circumference 32.4 cm. Scrotal circumference was highly correlated (0.91) with testicular volume and also with ejaculate volume (0.38). The correlation of 0.92 between scrotal circumference and testis volume was similar to the values of 0.92-0.95 reported by Hahn *et al*. (1969).

Bhosrekar *et al*. (1978) reported that testicular volume of the Holstein – Freisian and Jersey bulls by water displacement method was 736.3 (ml) and 694.7 (ml) respectively. Narasimharao *et al*. (1993) reported paired testicular volume 356, 263 (cm^3) at the age of 36.1- 42 and 30.1 – 36 months respectively in Murrah bulls.

Rana and Bilaspuri (1999) reported that testicular volume by using formula 443.7 (cm^3) in Murrah bulls. Pant *et al*. (2003) reported that testicular volume by using formula is 604.8 (cm^3) in Murrah bulls.

2.2. Reaction Time

Almquist and Hale (1956) emphasized the importance of study of sexual behaviour of bulls and reported high reliability of reaction time as a measure of

sexual activity. Hafez and Darwish (1956) stated that most objective measurements of sex drive was reaction time.

Kushwaha *et al*. (1955) reported 28.3 and 74.8 seconds as reaction time in first and second collection respectively, taken in rapid succession in Murrah bulls, whereas, Tomar *et al*. (1966) reported average reaction time of 79 seconds in same species. Kodagali (1967) and Nema (1982) recorded average reaction time of 180 seconds and 116±6.93 seconds in Jaffrabadi and Surti buffaloes, respectively.

Mukherjee and Bhattacharya (1952) reported that reaction time was not associated with semen quality. However, Kalev and Venkov (1959) reported positive correlation between reaction time and semen quality, whereas, El-Chahidi *et al*. (1980) reported that reaction time was negatively correlated with volume, percent live and abnormal spermatozoa in Egyptian buffaloes. Reaction time was significantly higher during summer than winter and rainy seasons among all the breeds.

Sexual behaviour is affected by a wide variety of factors of which age, season, endocrine constitution, frequency of collection, teaser practices followed at artificial insemination centers are the predominating (Baker *et al*., 1953; Bhatnagar *et al*., 1955; Hafez and Darwish, 1956 and Tomar *et al*., 1964). The buffalo bulls with higher sex drive are not influenced by changes in sex stimuli or time of collection as reported by Prabhu (1967). In a study, Belonozhkin (1977) used bulls as teasers for other bulls during semen collection and found a significant adverse effect of ejaculate volume, sperm concentration and ejaculation time when their own semen was collected after this.

Tomar and Gupta (1971) reported that reaction time in Murrah buffalo bull was 2.5 to 3.3 minutes. Situmorang, and Sitepu (1991) reported reaction time in swamp buffalo was 150 to 190 seconds.

Bhosrekar *et al*. (1992) reported reaction time in the age group of below 36 month and above 36 month in the winter and summer season was 70.15±3.049, 73.88±3.57 and 80.00±12.64, 71.88±2.28 second respectively. Tomar and Singh (1996) reported that mean reaction time in Murrah bulls was 66.1 seconds.

Sahu (1996) reported reaction time in Murrah bull semen was 139±11.62 seconds. The difference in reaction was highly significant ($p<0.01$) between bulls but non significant between age groups.

Mandal and Tyagi. (2004) reported that reaction time in young & adult Sahiwal bulls was 19.61±2.89 and 48.73±6.08 seconds respectively there was significant ($p<0.05$) difference between young and adult bulls.

2.2.1 Ejaculate Volume:

Volume of semen is an important characteristic for extensive utilization in A.I. Ejaculate volume varies from breed to breed and within the breed from to bull to bull (Rao *et al*., 1996). In general the volume increases with the age and the body size of the bull and changes with it's general reproductive health, vigour and the frequency of use.

The average volume of semen ejaculated by Murrah bulls ranged from 2.78 to 5.0 ml during 4 to 7 years of age (Tomar *et al*., 1966; Singh *et al*., 1967; Dugwekar, 1968; Singh *et al*., 1983; Tuli, 1984; Sekharan and Rao, 1986; Ganguli, 1988; Rattan, 1988; Tiwari *et al*., 1988; Narsimhrao *et al*., 1991; Rahman *et al*., 1991 and Tomar and Singh 1996).

Gopalakrishna and Rao (1978) reported that average volume of semen per ejaculate was in the months of March, April, May, June, July were 3.03±0.70, 2.68±0.62, 2.14±0.73, 2.47±0.78, 3.08±0.91 ml respectively. Bhavsar *et al*. (1986) reported ejaculate volume in Murrah bulls during hot, wet, and cold 3.56±0.19, 4.09±0.23, 3.85±0.23 ml. Similarly Sagdeo *et al*. (1991) reported ejaculate volume 2.2, 2.36 and 3.12 ml in winter, summer and monsoon respectively.

The quantity of semen usually does not exceed 5.0 ml in Indian buffalo bulls, through a volume of 13 ml was reported from Murrah bulls at Indian Veterinary Research Institute (Perry, 1969). Seasonal variation in semen volume has been observed by Pangawkar (1968) as it was 2.41±0.60 ml (spring), 2.88±0.35 ml (summer) seasons. Narsimharao *et al*. (1991) found non significant effect of season on ejaculate volume, whereas Sagdeo *et al*. (1991) reported significant difference in volume between seasons and between bulls in all seasons. According to Suryaprakasham *et al*. (1993) the mean ejaculatory volume increased parallel with body weight till 6.5 years of age followed by gradual decrease.

Sahu (1996) reported overall mean ejaculate volume was 2.52±0.10 ml. Semen volume differed significantly ($P<0.01$) between bulls but non significant between age group.

Maurya and Tuli (2003) reported ejaculate volume 3.45±0.25 ml in Murrah bulls. Rana and Dhami (2004) recorded ejaculate volume 7.03±0.44 and 6.36±0.33 ml in Gir and Jafarabadi bulls.

2.2.2 Colour

Iyer (1952) reported milky white colour and consistency of semen in Murrah bulls. Mahmoud (1952) and Ganguli (1988) observed it as milky white with very light bluish tinge. Kodagali (1967) found it as thick milky and scored 3.0 (in 0 to 5 scale) for colour and consistency of semen in Jaffrabadi bulls. Hukeri (1969) opined that in majority of buffalo bulls (85%) semen was milky white and in few (15%) it was creamy white in colour and consistency. However, Eusebio (1977) recorded creamy white colour and consistency of semen in Murrah bulls. Chaudhary and Gangwar (1978) scored buffalo bull semen as 2.48±0.32 (in 0 to 3 scale) for consistency. Sahu (1996) repoted breeding Murrah bull semen ranged between milky to thick creamy. Overall mean consistency of breeding bull semen was 3.49±0.07 on 1 to 4 scale.

2.2.3 Mass motility

The physical activity, which is important at certain stages of sperm transport in female reproductive tract (Lightfoot and Restall, 1971) is the basis of several methods of semen evaluation. Motility has long been used and identified as livability and fertilizing ability of sperm.

Motility is graded under low power (10x) of microscope according to the mass activity of spermatozoa. Mass motility is assessed by graded estimates of the vigor of swirls and wave formation in undiluted semen as seen under the microscope. Some workers recoded the mass spermatozoal activity in Murrah bull semen as 2.42±0.01 to 2.65±0.069 in 3 point scale (Prabhu and Bhattacharya, 1954; Chauhan, 1972; Chaudhary and Gangwar, 1978) and 3.0 to 3.61±0.15 on 4 point scale (Dugwekar, 1968 and Tuli, 1984). Bhosrekar *et al*. (1992) reported

mass motility in Murrah bulls 4.76±0.037, 4.57±0.054, and 4.73±0.042 in rainy, winter and summer season at 0-5 point scale.

Sahu (1996) reported the overall mean of mass motility was 3.83±0.04 on the 0 to 4 scale.

2.2.4 Initial Progressive motility

The estimate of mass motility is not very precise. Some percentage of spermatozoa weakly motile may be exaggerated under the influence of actively motile spermatozoa present in it. A range of 60.00 percent to 83.60±0.83 percent individual spermatozoal motility has been reported by various workers in Murrah bulls (Dugwekar, 1968; Pangawkar, 1968; Bhosrekar and Nagarcenkar, 1973; Eusebio, 1977; Gopalkrishna and Rao, 1978; Singh *et al*., 1983; Tuli, 1984 and Nath *et al*., 1991). Radev *et al*. (1967) scored individual motility as 88.80 percent in Egyptian and 89.90 percent in Indian buffalo bull semen. Datta *et al*. (1991) opined that sperm motility and live sperm count did not affect significantly by different methods of glycerolization. Sagdeo *et al*. (1991) reported that mass activity initial motility differences between seasons and between bulls were significant in Surti buffalo semen.

Sahu (1996) reported initial progressive motility in Murrah breeding bulls was 78.54 ± 0.30 percent. The difference in initial progressive motility was non significant between bulls.

Maurya and Tuli (2003) reported initial progressive motility 60.75±4.96 % in Murrah bull semen.

2.2.5 Sperm concentration

Semen samples vary in respect of the number of spermatozoa found per unit volume. Production of spermatozoa is a continuous process in sexually mature bulls, Spermatozoa are stored in epididymis and during the process of ejaculation, depending on the frequency and degree of excitement, and spermatozoa are diluted with seminal plasma.

The concentration of spermatozoa varies with the sexual development and maturity of the bulls, with the feeding regime and with the reproductive health and size of the testis, season of the year and different geographical localities (Salisbury, *et al*., 1985).

Sperm concentration in buffalo bull semen is relatively low than in cow bull semen. The sperm concentration varied between 631 to 1034 million sperm per ml in Murrah bull semen (Sengupta *et al*., 1963). Some other workers reported it to range between 220 to 1740 million/ml in Murrah bull semen (Tomar *et al*., 1966; Singh *et al*., 1967; Hukeri, 1969; Bhosrekar and Nagarcenkar, 1973 and Tuli, 1984). A significantly negative correlation between ejaculate volume and sperm concentration was cited by Shrivastava *et al*. (1979) in bull semen. Chaudhary and Gangwar (1978) reported that average concentration of spermatozoa per ml of ejaculate was 1160.00±60.00 millions. Singh *et al.* (1983) reported that average concentration of spermatozoa per ml of ejaculate was 1168 ±60.66 millions. Sekharan. and Rao. (1986) reported that average concentration of spermatozoa per ml of ejaculate was 1102±22.66 millions.

Sahu (1996) reported sperm concentration in the breeding Murrah bulls was 1311.18±38.36 million/ml. The difference in semen production between bulls was non significant.

Maurya and Tuli (2003) reported initial progressive sperm motility 60.75±4.96% in Murrah bull semen.

2.2.6 Percent live spermatozoa

Vital staining technique was used for the last three decades and it was popularly used for differentiating dead sperms from the live ones. Lasley *et al*. (1942) used opal blue staining technique on bull semen to differentiate live from dead sperms. Prabhu and Bhattacharya (1951) used the method advocated by Lasley *et al*. (1942) with slight modification. They found an average of 22.64% and 17.21% of dead spermatozoa in the first and second ejaculates respectively, in buffalo semen.

Swanson and Herman (1944) found significant ($p<0.01$) linear relationship ($r=0.84$) between conception rate and viability.

Madden *et al*. (1947) found no significant differences in percentage of live sperms between semen samples regardless of the fertilizing capacity.

Prabhu and Bhattacharya (1951) reported an average of 19.92 percent dead spermatozoa in Indian buffalo bull semen.

Ortavant *et al*. (1952) differentiated live and dead spermatozoa in the bull semen using a stain containing 4% aniline blue and 1% eosin dissolved in phosphate buffer (pH 6.78). The percentage of dead spermatozoa was least when smears were stained at 28°C, and the counts made 30 seconds after staining.

According to them, the optimal temperature for drying the smears was 28°C to 30°C.

Cupps *et al*. (1953) found a negative correlation ($r = -0.051$, $p<0.01$) between the percentage of dead sperms and conception rate, based on 30 to 60 day non-returns.

Hafez and Darwish (1956) concluded that percentage of live sperm were not significantly affected by successive collections in Egyptian buffalo bulls and they got 80.1 as the mean value for percentage of live sperms. Cumming (1954) reported a downward trend in fertility with a decrease in percentage of live sperms, and the rate of decline in fertility increased as the content of live sperm decreased.

Tomer *et al*. (1966) found that in Hariana bulls percentage of live spermatozoa varied significantly between months ($p<0.01$), and also between seasons ($p<0.05$), whereas, in Murrah buffalo bulls, a similar trend was obtained in spring and summer. Between seasons variation in either species was not significant.

Kodagali (1967) found that percentage of live spermatozoa in Gir and Jaffrabadi buffalo bulls to be 80 and 79 respectively.

Mukherjee and Kumar (1971) reported that percentage of dead spermatozoa were not significantly correlated to conception rate in Hariana bulls. Whereas, other workers have reported the average live spermatozoal percentage of 84.51 to 92.6 percent in Murrah bull semen (Gopalkrishna and Rao, 1978; Singh *et al*., 1983; Tomar and Gupta 1971; Sekharan and Rao 1986; Tiwari *et al*., 1984; Datta *et al*., 1991; Rahman *et al*.1991; Dhami and Kodagali 1988) in this breed.

Tomar and Singh (1996) reported that live percentage in Murrah bulls were 73.69±0.12.

Sahu (1996) reported overall mean live sperm percentage of Murrah bulls was 85.25±0.60 there was no significant difference in live sperm percentage between bulls.

Maurya and Tuli (2003) reported live sperm % in Murrah bull semen was 70.23±2.79%.

2.2.7 Percent abnormal spermatozoa

The sperm abnormalities could be morphological or deviations from staining reaction. Spermatozoal abnormalities are generally studied in terms of head, neck, midpiece and tail abnormalities. A good semen sample does not contain more than 20% abnormal spermatozoa (Anderson, 1946). Tomar *et al*. (1966) observed that there was no seasonal variation in the abnormal percentage of live sperms.

The morphology of spermatozoa is one of the important factors in evaluating semen. While studying spermatozoa for morphological abnormalities, Blom (1944, 1945 & 1950) differentiated between primary and secondary causes. The primary morphological abnormalities were caused by aberrations during spermatogenesis, while secondary abnormalities were caused by aberrations at the subsequent developmental stages. Usually, secondary abnormalities could be due to faulty or incomplete ejaculation, thermal shock, genital diseases, or improper handling of the ejaculates. The average proportion of primary abnormal forms, ranged from 4.6 to 10.6% (Blom, 1950., Anderson, 1939., 1941) and it was

generally believed that they should not exceed 15 to 20% (Blom, 1950., Lagerlof, 1936).

The types of abnormalities commonly encountered in the buffalo spermatozoa were similar to those observed in the bull and may involve head, midpiece, and the tail region. It was not clearly known how each type of abnormality affects the fertilizing ability of the spermatozoa. However, it has been generally accepted that a high percentage of abnormal spermatozoa indicate poor quality of the semen.

As regards to the percentage of abnormal spermatozoa in buffalo semen, the findings were variable and this variation could be due to the difference in the techniques of collection employed by them. Breed differences and the quality of the bull of the bull used could be other influencing factors on the evaluation of these characteristics.

Kushwaha *et al*. (1955) observed that in Indian buffaloes the percentage of abnormality varied from 2.7 to 14.4 with an average of 6.6. Prabhu and Bhattacharya (1951) reported a low percentage of abnormality of 4 to 5. Bhattacharya (1965) based on the finding of Roy *et al*. (1958) reported the abnormality as high as 17.2% in buffalo bulls. In the Egyptian buffaloes, Hafez and Darwish (1956) reported an average of 21.2% abnormal spermatozoa, with a range of 15 to 32%. Madatov (1956) found the average percentage as low as 3 in buffaloes (Russia) with a range of 1 to 11.5%.

Blom (1944) found peculiar multiciliate cells (medusa formation) in certain semen samples of bulls at a frequency of about 1 per 10000 spermatozoa examined from all bulls. These medusa formations were identified as detached

fragments of the ciliated epithelial cells of the efferent ducts of testis. The cells stained intensely and were about the size of spermatozoan head and also had 10 to 30 long filiform or brush-like projections. Blom (1950) stated that spermatozoal abnormalities were due to disorders in the spermogenic epithelium, or to non-physiological conditions affecting spermatozoa after they have left the spermogenic epithelium.

Tomar *et al*. (1966) and Bhattacharya *et al*. (1978) reported abnormal sperms as 17.3 to 21.5 percent in Murrah bull semen. Gopalkrishna and Rao (1978) recorded 18.88 percent abnormal spermatozoa involving 7.44% head, 1.46% midpiece, 1.53% percent protoplasmic droplets and 2.04% percent loose head abnormalities in Murrah bull semen.

Rao and Rao (1978) reported that difference in the incidence of total spermatozoal abnormalities was not significant ($p<0.01$) between Ongole bulls but same was significant ($p<0.01$) between the months. It was opined that elevated environmental temperature during summer impairs testicular functions and lead to increased sperm production with abnormal morphology.

Age of buffalo bull had a significant effect on sperm abnormalities, abnormalities was maximum in age group below 3 years, then declined and again increased after 5 years of age (Saeed *et al*., 1988).

Luthra and Mariony (1995) found that total abnormalities were highest during summer (29.47%) which differed significantly ($p<0.01$) from spring (19.0 %) and winter (19.69%) seasons in Holstein Friesian bulls.

Maurya and Tuli (2003) found overall abnormal sperm percentage in Murrah bull semen is 19.08±2.04 and it increases upto 31.24±2.29 after 3 hours

quality based thermal resistance of filtered and non filtered frozen-thawed Murrah buffalo semen incubated at 37^{o} C.

2.2.8 Percent intact acrosome

Acrosomal studies are more highly correlated with fertility (Saacke and White, 1972).detachment of acrosome results into decrease in ATP and loss of intracellular proteins responsible for fertilization.

Gopalakrishna and Rao (1978) reported that 7.44±1.25% acrosomal abnormalities in Murrah bull semen

Narasimha Rao *et al*. (1989) found 16.3% acrosomal abnormality in neat semen which increases to 47.5% in incubate Murrah semen. In 16.3 %, 8.8 % was detached 0% was swollen, 3.0% ruffled and 1.5% was in separating condition. Whereas in incubated semen detached acrosome increased upto 21.2%, Swollen spermatozoa increases 0 to 8.6 %, Ruffled spermatozoa increased upto 13.4%.

Singh *et al*. (1992) reported 12.37±1.92% acrosomal abnormality in fresh semen of Murrah bulls and it did not varies significantly ($p<0.05$) between bulls

Maurya and Tuli (2003) reported 14.85±1.10% abnormal acrosomal sperm in fresh semen sample, and its increases upto 48.14±2.47% after 3 hours quality based thermal resistance of filtered and non filtered frozen-thawed Murrah buffalo semen incubated at 37^{o} C.

2.3 Cryopreservation of Murrah Bull Semen

The success of an A.I. programme in any species is intimately linked with the effective prolongation of fertile life of spermatozoa, under in-vitro storage condition problems related to semen characteristics and sexual behaviour of

certain percentage of Murrah bulls are known. Despite that 40 and more years that have passed since the first success in Cambridge in freezing bull semen, it is probably true to say that no more than 50% of spermatozoa currently survive the freeze thaw process.

2.3.1 Frozen storage of semen: Basic aspects

A miraculous success in semen cryopreservation has been achieved after Polge discovered glycerol as a cryoprotectant. The influence of this technology upon the animal breeding industry especially dairying has been profound. However, the remarkable success achieved with the taurine bull semen has not been matched with other mammalian species (Holt, 2000). The basic techniques of cryopreservation established in the early 1950s have now been improved substantially through the research thereafter (Watson, 1990 a; Holt, 2000) to address the problems associated with poor freezability of the semen of some animal species and breeds.

The ultimate goal of semen preservation is to obtain pregnancies after artificial insemination comparable to those obtained after natural mating. The freezability (Post-thaw survival of cryopreserved semen) is known to vary between species, breeds and among individual males within the same breed. The recent progress in semen preservation has been limited as a result of empirical methods of optimization as revealed by various studies on post-thaw characteristics of Murrah bull semen.

2.3.2 Post-thaw Changes in Quality of Semen

Many tests have been employed for assessing a range of structural and functional aspects of spermatozoa. However, as of now no single test reflects its entirety in producing the satisfactory result towards prediction of potential fertility.

Cryopreserved spermatozoa have a poorer fertility than fresh spermatozoa which partly can be explained from the fact that the percent motility and liveability is reduced by about fourty percent or even more during freezing and thawing. This can not be the complete explanation since even when equal numbers of motile spermatozoa are inseminated intracervically, the cryopreserved semen has been reported to be inferior (Shanon, 1978).

Sagdeo *et al*. (1991) obtained best freezability during winter (48.74%) followed by summer (36.44%) and monsoon (14.82%) seasons in Surti buffalo bulls, Whereas, Bhosrekar *et al*. (1992) found rainy season and winter seasons as the most satisfactory seasons for freezing of buffalo semen.

2.3.3 Prefreeze Motility

Assessment of motility at prefreeze stage, after cooling of semen at 5° C and subsequent equilibration at this temperature is considered as prefreeze motility. It is an indicator of the activity of spermatozoa just prior to its freezing. Prefreeze motility has been reported to be significantly correlated with post-thaw motility (Kumar, 1989, Dhami, 1992).

2.3.4 Post thaw motility (PTM)

Motility of freshly ejaculated semen sample is not a good indicator of either fertility or the ability of sperm cells to survive freezing process. Once frozen semen has been thawed it must be kept at a constant temperature until it is inseminated, in order to avoid cold shock (Maksinov and Korovko, 1973). Semen samples vary greatly in the percentage of their cells that survive freezing from bull to bull. The accurate evaluation of post-thaw percent progressively motile sperm is of great importance, as it establishes the optimum dilution rate for each bull.

The mean and maximum post thaw motility obtained in Tris-egg yolk-citrate dilutor was 38.60±1.34 and 45.41±1.58%, respectively, in Surti buffaloes (Roy and Ansari, 1973; Sharma *et al*., 1979; Vasanth, 1979; Singh Sall et. *al*., 1980 and Chauhan *et al*., 1991). However, 42.97±1.04 to 65.92±3.95% post thaw motility was obtained by various workers in buffalo semen (Shetti, 1981., Abhi, 1982., Phalak, 1985., Bhalde *et al*., 1991., Datta *et al*., 1991 and Nath *et al*., 1991). It was demonstrated by Abhi (1982) that loss in individual sperm motility after freezing was 23.5%, whereas, Sengupta and Sukhija (1988) described the same as 14.1 to 39.1 percent in Murrah bull semen. Some workers said that conception rate is highly influenced by post thaw motility in cattle (Pandit and Garg, 1983) and buffaloes (Jani *et al*., 1983 and Nath *et al*., 1991). The Post thaw motility did not vary in cattle and buffaloes (45.23±1.71 Vs 44.49±1.99 %) but in individual bulls within the breed/species differed significantaly ($p<0.01$) at different steps of semen processing (Dhami *et al*., 1991).

Sagdeo et al. (1991) concluded that post thaw motility was best in winter followed by summer and monsoon season, and it varied significantly between bulls but non significantly between seasons in Surti buffalo bulls.

2.3.5 Live Sperm Percentage

Some workers (Mixner and Saroff, 1954 and Maule, 1962) have explained that counting of live/dead spermatozoa by vital staining of frozen semen with glycerolated dilutor was not satisfactory, whereas, Rathore (1965) obtained good results with the stain in bull semen. However, a range from 60.76±0.68 to 68.75±2.87 percent live spermatozoa is reported by some workers (Bhalde *et al*., 1991., Datta *et al*., 1991 and Nath *et al*., 1991). The live sperm percent declined

about 10 percent (neat semen 78.75±3.20 Vs frozen semen 68.75±2.87) after freezing of buffalo semen (Nath *et al*., 1991).

2.3.6 Post-thaw Intact Acrosome

Researchers have reported that aging of or injury to sperm causes Acrosomal cap deterioration. Changes are first noted at the apical ridge but progress to the point where the entire acrosome is gone. Abnormal acrosome renders the sperm unfit for fertilization. Thus the spermatozoa, characteristics influencing acrosome become equally important. Acrosomal integrity is significantly correlated with fertility of frozen thawed semen (Saacke and White, 1972), as well as refrigerated semen (Singh *et al*., 1992) and is simpler and easier method to evaluate the effect of freezing on sperm. Sperm acrosomal cap facilitates the entry of the sperm into the ovum and the fertilization process may be impaired by abnormal acrosomes (Bedford *et al*., 1968). Besides the existence of a variety of acrosomal anomalies, alterations or injuries to acrosome occur the different stages of semen processing and storage depending upon the micro environment conditions around the sperm or the nature of sperm itself. Information on the nature and extent of acrosomal alterations in frozen buffalo spermatozoa is scare.

2.3.7 Abnormal Sperm Percentage

Dilution of semen did not affect spermatozoan structure (Saacke and Marshall, 1968 and Singh *et al*., 1991), but the sperm morphology was highly affected between equilibration period and 24 hours after freezing (Singh *et al*., 1991). A significant increase in total sperm abnormalities after freezing of buffalo semen (neat semen 14.45±1.49 Vs frozen semen 20.83±3.61) was reported by

various workers (Shetti *et al*., 1981, Hazarika *et al*., 1989 and Nath *et al*., 1991), whereas, about 9% (neat semen 18.91 Vs frozen semen 27.4 percent) increase in incidence of total sperm abnormalities in frozen semen of Holstein Friesian bulls was studied by Luthra and Mariony (1995).

Sahu (1996) reported abnormal sperm percentage in the breeding Murrah bulls was 16.90±0.84 including 5.24±0.59 major abnormalities and 11.67±0.82% minor abnormalities. Abnormalities were lesser in younger bulls than older one.

2.3.8 Hypo-osmotic swelling test

Drevius and Ericksson (1966) reported that the volume of spermatozoa increase due to osmotic uptake of water, the cell membrane takes on a more and more spherical shape, apparently without a significant enlargement of its area. The "flexible motor" apparatus of the tail is forced by cell membrane to bend and coil.

The integrity of sperm membrane is important for sperm metabolism and a desirable change in properties of the membrane is required for successful interaction between males and female gametes. The integrity and functional activity of sperm membrane is of fundamental importance in the fertilization process (Jeyendran *et al*., 1984). Hence the assessment of membrane function maybe a useful indicator of the fertilizing ability of spermatozoa. The cell membrane has the ability to permit transport of molecules selectively. On exposure of spermatozoa to hypo-osmotic condition, water molecules enter the spermatozoa in an attempt to reach osmotic equilibrium. This influx of water increases sperm volume and causes bulging of plasma membrane, giving a minimum surface to volume ratio. The sperm tail appers to be more susceptible to such hypo-osmotic conditions (Jeyendran *et al*., 1984).

HOST was developed by Jeyendran *et al*., (1984). They suggested its use in clinical evaluation of human sperm function. They reported a positive correlation between motility and swollen spermatozoa with male fertility especially with that of in vitro fertilizing ability of spermatozoa. Pandya *et al*. obtained similar results (1986). This Hypo-osmotic swelling test correlates well with the ability of spermatozoa to penetrate denuded hamster oocyte and presents a high correlation with the capacity of the spermatozoa to fertilize the ovum in vitro (Vander Ven *et al*. 1986; Avery *et al*., 1988). Insignificant correlation between sperm swelling and in vitro sperm fertilizing ability has also been reported (Vanden Saffle *et al*., 1992). Karmur *et al*. (2002) reported 62.30±1.99% hypo-osmotic positive in fresh semen and 27.86±1.63% hypo-osmotic positive in post thaw semen of Murrah bulls in March month. Similarly values are also reported by Merja (1997); Rana (2001) and Prasad (1999 a). Mandal *et al*. (2006) reported that 33.96±1.78 percent spermatozoa showed swelling response in hypo-osmotic solution of Frieswal bull.

2.3.9 Cervical mucus penetration test (CMPT)

Sperm penetration into cervical mucus has been successfully used as a test of fertility in human (Kremer 1965) and bull (Murase & Braun, 1990) semen. The technique involves microscopic examination of the movement of vanguard spermatozoa into cervical mucus. The different rate of penetration in the capillary tubes with cervical mucus containing the sperm of different distance makes evaluates sperm fertilizing ability. Quantitative information can be obtained by observing sperm penetration of cervical mucus contained in the capillary tube.

The *in-vitro* mucus penetration test was introduced to diagnose human male infertility by measuring the ability of human spermatozoa to penetrate cervical mucus of homologous species (Kremer, 1965., Reichman *et al*., l973., Katz *et al*. 1980., Aitkin *et al*. 1986., Pandya *et al*., 1986) or bovine (Mughissi *et al*. 1982., Takemoto et al., 1985). However, only limited information is available on the effective usage of this method for prediction of Murrah bull fertility (Dev *et al*. 1996).

Sperm velocity and density parameters have good correlation with sperm penetration in bovines (Murase and Braun 1990). They further suggested that adequate lateral head displacement is essential for sperm penetration in estrus mucus. Galli *et al*. (1991) reported linear correlation ($p<0.01$) between sperm penetration and acrosomal integrity ($r=0.53$). Sperm motility is one of the most important factor in determining the penetration through estrus mucus showed significant positive correlation ($p<0.05$) with sperm penetration distance (SPD) value (Dev *et al*., 1996) SPD value was greatly influenced by cryopreservation of semen and showed decreasing trend when frozen semen was used instead of fresh semen in bovines (Matousek *et al*., 1989., Okuda *et al*. 1988., Prasad *et al*. 1999b)

Kumar and Devanathan (1996 a) suggested that among three major parameters of semen quality i.e. post-thaw motility, presence of normal form of spermatozoa and acrosome intact sperms, motility had more significant contribution for sperm progression speed (SPD) in cervical mucus. The average speed was reported as 55.95±1.003 micron/second. Kumar and Devanathan (1996 b) further reported the sperm penetration distance of 45.17mm/30 minutes in

normal mucus sample collected from jersey cow. They observed that cervical mucus penetration test was influenced by the quality of the cervical mucus.

2.4 Effect of incubation (37^{o} C) on post – thaw seminal characteristics

Chinnaiya and Balakrishnan (1988) reported after prolonging thawing at 38^{o} C Visual motility, live count and acrosomal maintenance at interval of 0, 15, 30, 45, 60, 75 min. They reported Visual motility 49.1, 41.9, 41, 33.2, 27.4, 20.7% respectively. They also reported live percentage & acrosomal integrity 58.1, 52.0, 47.8, 43.9, 38.1, 33.8 % and 69.9, 68.3, 65.2, 64.8, 63.4, 62.1 respectively.

Dhami *et al*. (1991) reported post thaw motility of Murrah bull semen at post thaw 0 and 1 hour 44.49 and 10.61% respectively.

Taraphder *et al*. (2001) reported motility after 0, 1 hours, 2 hours after incubation on 37^{o} C . 44.45±1.30, 34.16±1.41, and 29.81±1.65 respectively.

Sharma *et al*. (2003) reported acrosomal disintegration at the end of 0 min, 30 min, 60 min and 120 min after thawing at 37^{o} C for 1 min 42.5±5.76, 47.67±3.6, 49.4±2.72, 52.67±2.65 % in Murrah bull semen.

Maurya and Tuli (2003) reported percent progressive sperm motility, percent live sperm, percent abnormal sperms and percent damaged acrosome quality based thermal resistance of frozen-thawed Murrah buffalo semen incubated at 37^{o} C at 0 hour, 1 hour, 2 hour and 3 hours. Percent progressive sperm motility decreased 30.25±2.09 to 4.67±0.28%. Percent live sperm decreased to 46.89 to 20.66±1.39%. Percent abnormal sperm increases to 24.41±1.50 to 31.24±2.29% and percent damaged acrosome increases to 44.13±2.63 to 48.14±2.47%.

CHAPTER-III

MATERIALS AND METHODS

The present work entitled “Seminal characteristics and freezability of Murrah bull Semen during spring and summer seasons” was conducted in spring (February-March) and summer (April-June) seasons at Central Semen Station Anjora Durg (Chhattisgarh) during 2007-2008. Durg is located at an altitude of 951 feet above the mean sea level, at a latitude of 21° 10' North and a longitude of 81° 16' East. The climate of this place is of tropical region and relative humidity ranges between 20 to 75%.

3.1 Experimental animals

Six Murrah bulls of 5 to 7 years age were the experimental animals for the study. The bulls were used routinely for semen collection and they were maintained under identical feeding and management regimes during the entire course of study.

3.2 Sterilization of articles

The following procedure was followed in washing and sterilization all the glass wares used for semen collection and processing.

- Washed thoroughly with soap water
- Rinsed twice in double distilled water
- Dried and sterilized in hot air oven at 160^{o} C for 1 hour.

Buffer solutions (20 min), rubber articles and artificial vagina were autoclaved at 10 lb. pressure at 110^{o} C for 10 min. The straws, buffer and stains were exposed to ultraviolet rays for one hour before use.

3.3 Scrotal circumference

Scrotal circumference was measured with the help of flexible cloth measuring tape around the greatest diameter of the testes and scrotum, after pushing the testes firmly into scrotum (Rana & Bilaspuri, 1999).

3.4 Scrotal volume

Scrotal volume was determined by water displacement method according to Rosenberg (1979). This was done by taking 3 liter plastic bucket filled up to the brim with warm (40^{o} C) water. The volume of water displaced, when the scrotal sac with contents was immersed in the bucket gives testicular volume.

Scrotal volume was also calculated by using the formula for volume of an ellipsoid, as per Love *et al*., (1991).

$$= 4/3(\pi\ abc),$$

Where

$$a = \text{thickness}/2,$$

$$b = \text{width}/2,$$

$$c = \text{length}/2.$$

Testicular Parameter such as length (mm) width (mm) and thickness (mm) was measured with help of divider as described by Singh and Pangawkar (1989).

3.5 Reaction time

It is a measure of libido. Reaction time is the time interval between first contact with the mating partner and the first attempt to mount. It was recorded in seconds with help of stopwatch (Rosenberg, 1979).

3.6 Collection of semen

Semen from buffalo bulls were collected in artificial vagina (40 cm. long and 6.5 cm in diameter) in which water at a temperature of 45^{o} C was filled. The

semen was collected over a male partner of the same species (Fig no. 1). Collections were taken during the morning hours between 7.00 to 8.30 A.M. before feeding. A practice of cleaning and washing the bulls was routinely followed. Immediately after collection, the tube containing semen were placed in a water bath at 37^{o} C and the samples were evaluated for various macroscopic (volume, colour and consistency) and microscopic (mass motility, initial progressive motility, percent live sperm, percent total abnormal sperm and percent intact acrosome) characteristics as described by Salisbury *et al*. (1978). Cervical mucus penetration test (CMPT) and Hypo-osmotic swelling test (HOST) were performed, as described by Kremer *et al*. (1965) and Jeyendran *et al*. (1984) respectively.

3.6.1 Sampling procedure

A total of 60 ejaculate in spring (February-March) and 60 in summer (April-June) were collected for the study. The ejaculate was divided in two parts. First part was evaluated, extended and frozen while small quantity in second part was used in cervical mucus penetration test (CMPT) and hypo-osmotic swelling test (HOST).

3.7 Seminal characteristics

Various seminal characteristics are examined in order to predict the fertility of the bull. However, none of the semen characteristics singly can predict the quality and fertilizing ability of given semen sample quite accurately and precisely. A combination of these characteristics helps in selection of an ejaculate, having a higher freezability and /of fertility potential.

3.7.1 Ejaculate volume

The ejaculate volume was recorded in milliliter (ml) directly from the graduated semen collection tube.

3.7.2 Colour

The colour of semen was recorded visually. The colour was graded as milky white, creamy white and watery. Numerical values were given to the colour for statistical analysis as under

Creamy white	:	2
Milky white	:	1
Watery	:	0

3.7.3 Mass activity

The mass activity of semen was recorded by placing a small drop of freshly collected neat semen on a clean grease-free, prewarmed glass slide at 37^{o} C and examined without coverslip, under low power magnification (10x) of a phase contrast microscope (Nikon, Japan) fitted with a thermostatically controlled warm stage. The mass activity of semen was graded on '0' to '5' scale based on the appearance of waves and swirls (Salisbury *et al*., 1978).

3.7.4 Initial progressive motility

The neat semen was extended at 37^{o} C with Tris buffer to an extent that approximately 15-20 spermatozoa were visible under a visual field of microscope (Zemjanis.1970). The initial motility of freshly diluted semen were assessed after covering the semen drop on slide with a thin cover slip at 37^{o} C under a high power magnification (40x).

3.7.5 Sperm concentration (million/ml)

The concentration of spermatozoa in neat semen was determined by the digital photometer (Accucell, IMV Technology).

3.7.6 Percent live spermatozoa

The percentage of live spermatozoa was estimated by differential staining technique using Eosin-Nigrosin stain (Campbell *et al*., 1953). The smears were prepared in duplicate, after gentle mixing a small drop of neat semen with 4 drops of stain on a clear grease-free slide at 37^{o} C.

At least 200 spermatozoa were counted under the oil-immersion objective (100x) of a phase contrast microscope for estimating the percentage of live (unstained) spermatozoa. The stained (eosinophilic) as well as partially stained spermatozoa were classified as dead.

3.7.7 Percent abnormal spermatozoa

Percent abnormal spermatozoa was determined using rose bengal stained smear. The incidence of proximal protoplasmic droplets, loose heads, mid piece and tail abnormalities were estimated according to Rao (1971).

The composition of stain includes

Rose Bengal powder	:	3 gm
Distilled water	:	99 ml
Commercial Formalin	:	1 ml.

Semen was diluted with 2.9% Sodium citrate buffer (1:10) at 37^{o} C and smear was prepared in duplicate in clean grease-free slide. Smear was immersed in stain for 10 minutes, after that it was washed in tap water.

At least 200 spermatozoa were counted under the oil-immersion objective (100x) of a phase contrast microscope for estimating the sperm abnormality.

3.7.8 Percent intact acrosome

The acrosomal integrity (percent abnormal acrosome) based on acrosomal damage in fresh and post-thawed semen was studied using Giemsa staining (Watson, 1975).

a. Giemsa solution [E. Merck (India) Ltd.] was used as stock solution

b. Sorensen's 0.1 M phosphate buffer (pH 7.0) solution

Solution – A. 0.1 M Potassium dihydrogen phosphate solution :

Potassium. dihydrogen phosphate (Anhydrous) : 13.6 gm.

Double glass distilled water : 1000 ml.

Solution – B. 0.1 M disodium hydrogen orthophosphate solution :

Disodium hydrogen phosphate : 14.198 gm.

Double glass distilled water : 1000 ml.

Sorenson's 0.1 M phosphate buffer (pH 7.0) was prepared by mixing 17ml.

Solution 'A' and 33 ml. of Solution 'B' and pH was 7.0.

c. Hancock's Fixative

Sodium Chloride	:	10 gm
Sodium bicarbonate	:	0.5 gm
Formalin	:	125 ml
Distilled Water	:	1000 ml

d. Giemsa working solution

Stock Giemsa Solution	:	3.0 ml
Sorensen's 0.1 M phosphate buffer (pH 7.0)	:	2.0 ml
Double glass distilled water	:	35 ml

e. Procedure

1. Neat semen was diluted with 2.9% Sodium Citrate dehydrate solution (1: 5 to 1: 10 at 35^{o} C).
2. Smears were prepared (in duplicate) on clean microscopic slides and air dried quickly.
3. The smears, immediately after drying were fixed in Hancock's fixative for 15 min.
4. The smears, were then washed in slow running tap water for 20 min.
5. After rinsing briskly in distilled water, the smears were stained in Giemsa working solution for 100 min.
6. The smears were then removed from the stain and rinsed quickly in distilled water, air dried and mounted in DPX mountant (BDX-Glaxo Laboratories, India).

The acrosomal abnormalities were classified as knobbed, Swollen, Denuded, Incomplete and Ruffled.

3.8 Cervical Mucus Penetration Test (CMPT)

The cervical mucus penetration test was carried out by using the method of Kremer (1965) and Matouseket *et al*. (1989) with slight modification i.e. capillary tubes were kept in vertical position (Prasad *et al*., 1999 b) instead of horizontal position.

3.8.1 Collection of estrous mucus

Cervical mucus was collected from she buffalo during standing estrus. After securing the animal properly, rectum was evacuated by back racking and then perineal region and vulval lips were cleaned thoroughly and disinfected with 70% alcohol. The genital tract was gently massaged by milking action per-rectally, to discharge estrous mucus from the vulva and collected in a clean and sterilized petridish aseptically. The mucus was observed for fern pattern and presence of any infection by white side test (Pateria and Rawal, 1990).The cervical mucus having typical fern pattern and free from infection was stored at -20^0 C for further use in CMPT. The mucus was thawed at room temperature for at least 2 hours

Loading of the capillary tube and incubation

The stored cervical mucus was emptied into a petri dish and was warmed to room temperature. The warmed cervical mucus was then aspirated into the capillary tube by drawing plunger of the syringe attached to it with the tubing of scalp vein. The trailing mucus was cut with scissors leaving a small amount protruding from the filled end of the tube. One end of the filled tube was sealed with paraffin while the other end left free.

- The tubes were then allowed to stand for 5 minutes at 37^o C. Only non-heparinized hematocrit capillary tubes were used for the test.
- About 0.5 ml semen sample was taken in a 2.0 ml, micro centrifuge tube (with a hole made on its cork lid) in duplicate so that the free end of capillary tube remained dipped in the semen just above the bottom of the tube.
- These tubes were incubated at 37^o C for 60 min.

- Capillary tubes were removed from the micro centrifuge tube. Outer surface of the capillary tube was wiped, cleaned and placed on a graduated glass slide.
- The distance (in mm) travelled by the vanguard spermatozoa was measured under low power (20x) of a phase contrast microscope. The mean of the two observations made in duplicate were recorded. Same procedure was followed for the post-thawed semen.

3.8 Hypo-osmotic swelling test (HOST)

HOST was carried out according to the method used for human spermatozoa described by Jeyendran *et al*. (1984).

Table 1: Composition of solutions for HOS test

	HOS test solution	Control solution
Fructose (g)	1.351	5.4
Trisodium citrate (g)	0.735	2.94
Millipore water to	100ml	100ml
Osmolarity	150 m Osm/kg	300 m Osm/kg

- 1.0 ml of hypo-osmotic solution was taken in a small test tube.
- 0.1ml of semen was added and mixed well. The sperm suspension was incubated at 37^{o} C for 60 min.
- After incubation a drop of eosin-y solution was added, a small drop of suspension from the bottom of the tube was placed on clean grease free glass slide and covered with cover slip.
- The slides were examined under the high power magnification (400 x) of a phase contrast microscope.

- A minimum of 100 spermatozoa were counted and the different types of swelling patterns recorded.
- Similar procedure was followed for post thaw cryopreserved semen.

3.8.1 Interpretation : One hundred spermatozoa were classified in 4 different classes according to the presence of following tail swelling patterns :

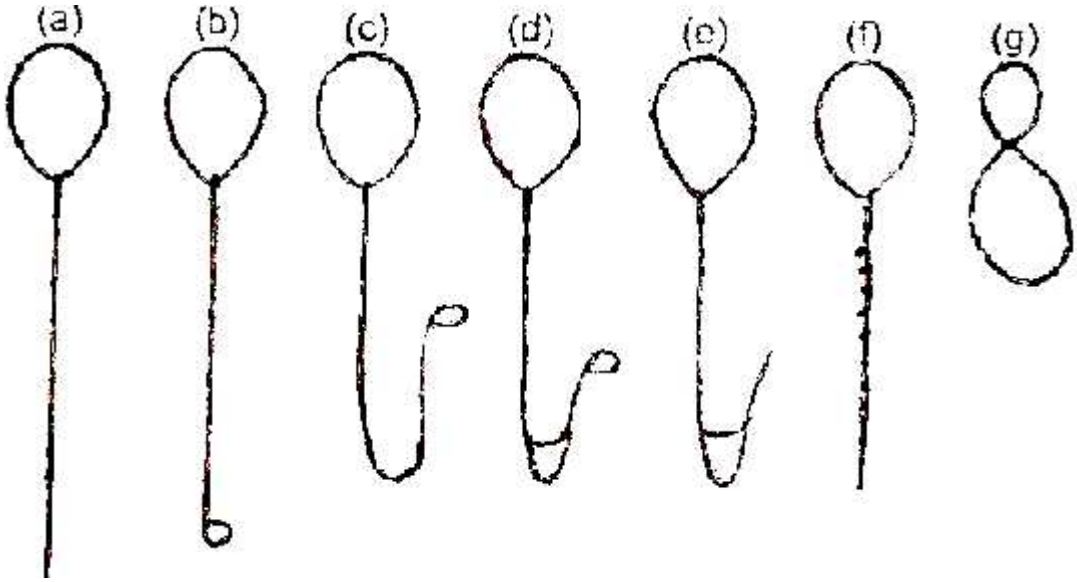
Pattern A : No swelling, no membrane reaction (Fig. a)

Pattern B : Swelling of the tip of the tail (Fig. b)

Pattern C : Different types of hair-pin like swelling of the mid piece (Fig.c to f)

Pattern D : Complete tail swelling (Fig. g)

Cells displaying pattern b, c, and d were considered positive for the HOS Test.



3.9 Dilution of Semen

3.9.1 Preparation of Dilutor: For preparation of Tris-Fructose-Yolk-Glycerol (Davis *et al*., 1963) dilutor Millipore water and analytical grade reagents were used.

Composition of dilutor / liter

Tris (Hydroxymethyl) amino methane (AR/GR)	:	24.22 gm
Citric acid mono hydrate (AR/GR)	:	13.60 gm
Fructose (AR/GR)	:	10.00 gm
Glycerol (AR/GR)	:	64.00 ml
Penicillin G-Sodium	:	10.00 lac IU
Streptomycin	:	1.00gm
Egg yolk	:	200 ml
Millipore water	:	736 ml to make1 liter.

3.9.2 Preparation of Buffer

- 600 ml Millipore water was measured in a conical flask (2.0 liter capacity).
- 24.22 gm TRIS was added to water and dissolved by gentle mixing.
- Citric acid 13.6 gm was added and dissolved.
- 10 gm Fructose was added and dissolved.
- Solution was poured into 1 liter measuring cylinder & volume was made 1 liter by adding 736 ml of Millipore water.
- Solution was then poured back into conical flask.
- 64.00 ml of Glycerol was added and mixed properly.
- Solution was autoclaved under 5 psi pressure for 20 min. and then cooled to room temp.
- Buffer was preserved in refrigerator.

3.9.3 Preparation of Extender

- 800 ml of buffer that was prepared is taken in a conical flask.
- 200 ml of freshly collected egg yolk was added to the buffer.
- 10 lack I.U. Benzyle penicillin and 1 gm. Streptomycin sulphate was added to the buffer, and it was mixed by magnectic stirrer using a sterilized teflon coated bar for 20 minutes at 34^{o} C.

3.10 Effect of duration of incubation at (37^{o} C) on post – thaw seminal characteristics

In present investigation effect of duration of incubation at 37^{0} C on post – thawed seminal characteristics at 15 minutes interval up to 60 minutes of incubation was studied for.

Motility

Live sperm percentage

Percent abnormal spermatozoa

Percent intact acrosome

Cervical mucus penetration test and

Hypo-osmotic swelling test

3.11 Effect of level of liquid nitrogen on post thaw motility

Straws were stored in liquid nitrogen container in which straws were immersed into liquid nitrogen upto ¾, ½, and ¼ level. The level of liquid nitrogen was maintained upto 120 hrs. The post –thaw seminal characteristic was evaluated at 24, 48, 72, 96 and 120 hrs of storage.

STATISTICAL ANALYSIS

The data, were analyzed through ANOVA one way, to find out statistical variation between the mean values of different bulls at different intervals of the observation period, Correlation (two tailed) between various parameter and paired t test for comparison of mean between seasons and different time interval for find out statistical variation between mean values of different bulls as per the standard procedure outlined by Snedecor and Cochran, (1967) using the software SPSS 10 for Windows.

CHAPTER-IV
RESULTS AND DISSCUSSION

4.1 Scrotal biometry

4.1.1 Scrotal circumference

Mean scrotal circumference in Murrah buffalo bulls was 33.41±0.33 (range 29.12 to 36.28) 33.65±0.34 (range 28.60 to 37.80) cm. in spring and summer seasons respectively. Scrotal circumference differed significantly ($p<0.05$) between bulls in both season but not between seasons (Table No 2).

It was suggested that a scrotal circumference of more than 32.0 cm was necessary for Holstein Friesian bulls to be classed as a potentially satisfactory breeder (Elmore *et al*. 1976). The testes produce potentially fertile spermatozoa and testosterone hormone. The bull testis is an elongated oval body which is 4 to 5 inches long and 2 to 2.5 inches wide at maturity (Salisbury and VanDemark, 1961).

Our findings are comparable with Narsimharao *et al*. (1993); Suryaprakasham *et al*. (1993); Rana and Bilaspuri (1999) and Pant *et al*. (2003) in Murrah bulls. However, Nema and Kodagali (1994) reported higher trance scrotal circumference in Surti buffalo bulls, which may be due to breed difference. Joshi *et al*. (1967) reported lower scrotal circumference in non-descript buffalo bulls.

4.1.2. Scrotal volume

Mean scrotal volume was 676.00±19.30 ml (range 470 to 890 ml) 656.38±18.77 cm^3 (range 460.53 to 880.24 cm^3) in spring.

Mean scrotal volume was 682.17±18.91 ml (range 472 to 907 ml) 665.96±18.72 cm^3 (range 454.24 to 895.28 cm^3). Significant ($p<0.05$) difference

in scrotal volume between bulls was recorded in spring and summer but not between seasons (Table no. 2).

Our findings are comparable with Pant *et al*. (2003) in Murrah bulls. However higher values were reported by Bhosrekar *et al*. (1978) and Mohanty *et al*. (1988). Whereas lower value was reported by Narasimharao *et al*. (1993) and Rana and Bilaspuri (1999).

4.1.2. Scrotal circumference and semen production

Scrotal circumference had positive correlation with ejaculate volume, concentration and live sperm during both seasons (Table no 3).

Our findings are similar to Nema and Kodagali (1994) and Pant *et al*. (2003).

4.1.3. Scrotal volume and semen production

Scrotal volume had positive correlation with ejaculate volume, sperm concentration and live sperm during spring and summer seasons (Table no 3).

Similar positive correlation between scrotal volume and ejaculate volume was reported by (range 0.40 to 0.50) Boyd and VanDemark, 1957; Hahn *et al*. 1969; VanDemark. 1986. Correlation coefficient was significant ($p<0.05$).

4.2 Reaction time

Mean reaction time in Murrah buffalo bull was 116.70±6.31 (range 59 to 287) and 123.71±7.53 (range 60 to 201) seconds during spring and summer seasons respectively. Reaction time did not differ significantly ($p<0.05$) between bulls during spring, while it differed significantly ($p<0.05$) between bulls during summer (Table no. 4). However there was non significant ($p<0.05$) difference in reaction time during spring and summer seasons.

The reaction time reported in the present study for the Murrah bulls was comparable to that reported by Tomar and Gupta (1971), Tomar & Singh (1996).

Higher value was reported by Situmorang & Sitepu (1991) and Kodagali (1967) in Jaffrabadi bulls. Lower value was reported by Kushwaha *et al*., 1955; Tomar *et al*., 1966 and Tiwari, 1984.

The discrepancy in reaction time may be attributed to the various factors like nutrition (Mixner, 1959), genotype and stressful condition like diseases (Hafez and Bonadonna, 1960 and Janowski *et al*., 1961) frequency of semen collection (Cunningham *et al*., 1967) age and environmental factors like temperature and managemental variations (Morrow, 1980). The significant ($p<0.05$) difference in reaction time between bulls during summer in present study indicate the individual variations for sensitivity to heat stress.

4.2.1 Reaction time and semen production

Reaction time had negative correlation with ejaculate volume, concentration and live sperm in spring and summer season (Table No 3). Our findings are similar to that of Sahu (1996).

4.3 Seminal characteristics of fresh semen of Murrah bulls during spring and summer

4.3.1 Ejaculate volume

Mean ejaculate volume was 3.83±0.25 ml. (range 1.10 to 14.00) and 4.02±0.22 (range 0.8 to 8.00) ml. during spring and summer seasons respectively. There was non significant ($p<0.05$) difference in ejaculate volume during spring and summer seasons. However significant ($p<0.05$) variation in ejaculate volume was recorded between bulls (Table No 5).

Gopalakrishna and Rao (1978) reported lower ejaculate volume in Murrah bulls during spring and summer seasons. Bhattacharya *et al*. (1978) reported higher ejaculate volume in Surti and Murrah bulls during spring season.

Similarly Gupta *et al*. (1978) reported lower ejaculate volume in Surti buffalo in spring season. Bhattacharya *et al*. (1978) reported higher ejaculate volume in Murrah bulls during summer season. These findings are in agreement with those of Kushwaha *et al*. (1955) and Tomar *et al*. (1966) who reported differences in the ejaculate volume of buffalo bulls due to seasons. Our findings are not with the agreement of Sengupta *et al*. (1963) and Bhattacharya *et al*. (1978).

Mann (1964) described that the ejaculate volume may differ greatly from bull to bull as it is dependent on the functions of accessory sex glands, which are androgen dependent for their physiological activities. However, the variation in semen volume may be due to degree of sexual excitement (Collins *et al*., 1951), nutritional status (Mukherjee and Bhattacharya, 1952), frequency of semen collection (Prabhu and Sharma, 1954), diseases (Mixner 1959), age (Singh *et al*., 1967) and season (Bhosrekar *et al*., 1980).

4.3.2 Mass activity

Mean mass activity in the present study was 3.40±0.10 (range 2 to 5) and 3.21±0.14 (range 0 to 5) percent during spring and summer seasons respectively. Mass activity between bulls differ significantly ($p<0.05$) in both seasons. However it does not differ significantly ($p<0.05$) between season (Table 5).

Mass motility reported in the present study for Murrah bulls was comparable to that reported by Dugwekar (1968) and Tuli, (1984) in Murrah buffalo bulls. Heuer *et al*. (1982); Vyawanare *et al*. (1989); Younis (1996) and Javed *et al*. (2000) reported lower mass activity in Nili-Ravi buffalo bull. Higher value was reported by Sahu (1996).

One of the reasons for higher score of mass motility in the present study may probably be due to less number of initially static semen samples (3 out of 120 ejaculates) as compared to other workers reporting 25 static ejaculates out of 125 semen samples (Tiwari, 1984).

Sengupta and Sukhija (1988) stated that occurrence of static ejaculate in buffalo might be due to an area problem linked with certain reasons and type of fodder fed to the bulls and opined that some non-dializable seminal factors known as anti-motility factors were responsible for this reversible phenomenon and some factors (Protein) in the dilutors may be neutralizing the effect on dilution. Furthermore, it was suggested to overcome this problem by collecting the ejaculates in the buffered dilutor directly for instant neutralization of the alleged anti-motility factors.

The variation in result may be attributed to due to difference in judgment of mass activity, may be due to difference in total number of observations made and climatic condition.

4.3.3 Colour

The colour of semen was recorded visually. The colour was graded as watery, milky white and creamy white (0 to 2). The colour of semen studied was actually the thickness of semen together with the colour of pigment. The colour of semen in Murrah buffalo bulls was milky to creamy in both seasons (Table 5). There was non significant ($p<0.05$) difference between the bulls during spring season (Table No 5). There was non significant ($p<0.05$) difference in semen colour between season (Table no 5).

In summer season mean for semen colour was 1.35±0.08 means milky to creamy and it varies from 0.60±0.16 to 1.70±0.15 means watery to creamy colour.

However there was significant ($p<0.05$) difference between bulls during summer season (Table No 5). Similar to present study Jainudeen *et al*. (1982) in swamp, Heuer *et al*. (1987) in Nili-Ravi buffalo bull reported milky-white coloured semen. The whitish or milky colour of semen, reported to give an estimated concentration of $5x10^5$ to $1x\ 10^6$ sperm/mm^3 (Settergren, 1982).

4.3.4 Sperm concentration

Mean sperm concentration in spring season was 1374.40±573.67 (range 600.40 to 2713.00) million/ml. Sperm concentration differ significantly ($p<0.05$) between bulls (Table 5).

Mean sperm concentration in summer season was 1419.94±88.84 (range 207.10 to 2872.00) million/ml in summer season. Sperm concentration also differed significantly ($p<0.05$) between bulls but not between season (Table 5).

Chaudhary and Gangwar (1978); Gopalakrishna and Rao (1978); Tomar & Singh (1996); Singh *et al*. (1983); Sekharan and Rao (1986) and Rahman *et al*. (1991) reported lower sperm concentration. However El-Wishy (1978), Raizada *et al*. (1988), and Terezinha *et al*. (1989) reported higher sperm concentration (1.65, 2.90, and 1.38 x 10^9/ ml) in buffalo bull.

Salisbury *et al*. (1985) described that spermatogenesis is primarily controlled by the activity of FSH from anterior pituitary gland and sperm concentration vary with sexual development and maturity of the bulls.

Such variations always are expected from workers working at different places with a variation in the animals selected, and which may be belonging to different age group. The no. of sperm per unit of ejaculate volume depends on the libido, sexual rest and frequency of ejaculation (Kumar, 1979) and hence the

variation in sperm concentration as reported by other workers might be due to above factors.

4.3.5 Initial progressive motility

Mean initial progressive motility was 83.91±0.65 (range 70 to 90) and 77.10±1.57 (range 50 to 90) percent in spring and summer seasons respectively. Initial progressive motility differed significantly ($p<0.05$) between bulls and between seasons (Table 5).

Our findings are in agreement with that reported by Gopalakrishna and Rao (1978) & Sekharan and Rao (1986). They also reported lower motility in summer season, which was also comparable with our findings. The difference may be attributed to different agroclimatic conditions. This could also be due to the variation in the seminal plasma composition, since it is known to affect the spermatozoan motility (Ganguly, 1988).

4.3.6 Hypo-osmotic swelling test

Mean hypo-osmotic swelling positive sperm was 52.68±0.95% (range 38 to 63) percent. Hypo-osmotic swelling sperm did not differ significantly ($p<0.05$) between bulls in spring (Table no 5).

Mean hypo-osmotic positive sperm was 50.24±1.31% (range 31 to 66) percent. Hypo-osmotic swelled sperm differed significantly ($p<0.05$) between bulls in summer season and it did not differed significantly ($p<0.05$) between seasons (Table 5).

The hypo-osmotic swelled positive sperm reported in the present study was lower to that reported by Mandal *et al*. (2003) and Karmur *et al*. (2002) while, Sivaramalingam (1994), Prasad *et al*. (1999 a) and Rasul *et al*. (2000) reported higher hypo-osmotic positive sperm in fresh semen of Murrah bulls.

Murrah buffalo bull spermatozoa showed morphological changes by coiling of the tail when subjected to hypo-osmotic solution. The tail coiling depends upon the ability of sperm membrane to allow passage of water in order to establish an equilibrium between extra and intra cellular fluid (Drevius, 1972). Transportation of compounds across the sperm membrane is an important biochemical process which is essential for sperm viability. Sperm motility depends partly on membrane transport (membrane integrity) and partly on other bio-chemical activities such as sperm metabolism (Jeyendran *et. al*., 1984)

4.3.7. Cervical mucus penetration test

Mean penetration distance was 26.18±0.66 (range 16 to 34) mm. in spring. Cervical mucus penetration distance did not differ significantly ($p<0.05$) between bulls (Table 5).

Mean penetration distance was 22.29±0.69 (range 14 to 34) mm. in summer. Cervical mucus penetration distance differ significantly ($p<0.05$) between bull and between ($p<0.05$) season (Table no 5).

The mean cervical mucus penetration distance in the present study for Murrah bulls are lower than that reported by Dev *et al*. (1996) and Kaur (1986).

Sperm motility, one of the most important factors in determining the penetration through estrous mucus, showed significant ($p<0.01$) positive correlation with sperm penetration distance value. This is in agreement with the observations of Sidhu *et al*. (1986) and Okuda *et al*. (1988). However, a few semen samples with low motility exhibited good penetration distance. This indicates that the kinetics and quality of progression of spermatozoa in estrous mucus is also important than merely the percentage of motile spermatozoa (Keel

and Webster 1988). Murase and Braun (1990) suggested that adequate lateral head displacement is essential for sperm penetration in estrous mucus.

A significant ($p<0.01$) correlation between live sperm count (before and after freezing) and sperm penetration distance values obtained in the present study was similar to result shown by Kaur (1986). This clearly indicates that only the live spermatozoa are able to penetrate the micelle of the estrous mucus. It can therefore, be inferred from this study that the sperm penetration test can provide more objective, although indirect, evaluation of semen fertility.

4.3.8 Live sperm percentage

Mean live sperm percentage was 75.87±1.12 (range 59 to 92) 67.02±1.19 (range 41 to 89) percent during spring and summer seasons respectively. Live sperm percent differ significantly ($p<0.05$) between bulls and also between season (Table no 5).

According to Herman and Madden (1953) the data on live sperm count can be used concurrently with motility score for assessment of semen quality. A fairly high percentage of dead sperm may not be apparent in the motility test because many of the inactive sperms move about passively by the impact of live sperm. Furthermore, a high percentage of live sperm is of little consequence if the sperm were showing only slow oscillatory movement. Hence, the live-dead count and the microscopic motility scores are probably more conclusive in assessing the relative fertility of bulls when they are used conjointly.

The percent live sperm for Murrah bulls in the present study was in close agreement with that observed by Tomar and Singh (1996) and Situmorang and Sitepu (1991) in Murrah bulls but higher than that reported by Tomar and Gupta (1971), Gopalakrishna and Rao (1978), Singh *et al*. (1983), Sekharan and Rao

(1986), Tiwari *et al*. (1984), Rahman *et al* (1991) and Sahu (1996) in this breed. The lower live sperm percentage as obtained in this study may be ascribed to the fact that all seminal ejaculates have been included in the study. While other workers have included only selective ejaculates.

4.3.9 Abnormal sperm percentage

Mean abnormal sperm in spring was 13.83±0.54 (range 7 to 21) percent. Bulls did not differ significantly ($p<0.05$).

Mean sperm abnormality in summer was 13.85±0.66 (range 5 to 28). Sperm abnormalities differed significantly ($p<0.05$) between bulls. However, there was non significant difference between seasons (Table no 5 & Fig no. 2, 3)

The present findings are in close agreement with the findings of several other workers in Murrah bull semen (Tomar et al., 1966; Eusebio, 1977; Gopalkrishna and Rao, 1978). However, Luthra and Mariony (1995) and Maurya and Tuli (2003) found higher morphological abnormal sperm.

Variation obtained in the sperm abnormalities might possibly be due to season and agroclimatic conditions (Saxena and Tripathi, 1985) and different techniques employed for studying the estimation of abnormalities (Rao and Rao, 1978).

4.3.10 Acrosomal integrity

Mean acrosomal damage was 14.80±0.54 (range 3 to 18) and 15.78±0.48 (range 9 to 25) percent in spring and summer seasons respectively.

There was significant ($p<0.05$) difference in acrosomal integrity between bulls and between seasons (Table no. 5 & fig no. 4, 5).

The percent intact acrosome for Murrah bulls reported in the present study is comparable to that of Gopalakrishna and Rao (1978). A higher concentration of

percent intact acrosome as compared to our findings had been noticed by Narasimharao *et al*. (1991), Maurya and Tuli (2003).

4.4. Seminal Characteristics of frozen semen of Murrah bulls during Spring and Summer

4.4.1 Pre freeze motility

Mean pre freeze motility was 77.83±0.63 (range 63 to 80) and 71.84±1.51 (range 45 to 80) percent during spring and summer seasons respectively. Pre freeze motility differ significantly ($p<0.05$) between bulls and also between season (Table no 6).

Prefreeze motility has been reported to be significantly correlated with post-thaw motility (Kumar, 1989, Dhami, 1992) as it is in this study.

4.4.2 Post-thaw motility (PTM)

Mean post-thaw motility was 64.83±0.68 (range 55 to 75) 58.64±1.34 (range 30 to 70) percent during spring and summer seasons respectively. Post-thaw motility differ significantly ($p<0.05$) between bulls and between seasons (Table no 6).

Our findings are in accordance with that of Haq (1966) and Pandit (1984) who also reported similar freezability of semen. No single attributes of semen can be relied upon for predicting fertility status of an ejaculate, however estimation of post thaw motility is increasingly being used as yardstick to assess the success of freezing technology and freezability of buffalo bull semen. Though a single sperm is able to fertilize the ovum but millions of sperm are required to counteract the uterine environment and to cross many hurdles to reach the site of fertilization. Thus giving more emphasis on post-thaw sperm recovery is an absolute necessity. It was opined by Rao *et al.* (1979) that the semen quality improved with age of the bulls and might be related to the function of the testes, whereas, Nath *et al*. (1991)

reported that the lower freezability of the semen was due to poor resistance of sperm cells against adverse effect of ultra low temperature in younger buffalo bulls.

4.4.3 Post-thaw percent live sperm

Mean live sperm was 61.45±0.99 (range 41 to 77) 56.43±1.15% (range 30 to 80) percent spring and summer season respectively (Table no 6). Bulls differed significantly ($p<0.05$) to each other in spring and summer season and also between seasons (Table no 6).

The live sperm percent dropped from 75.87±1.12 to 61.45±0.99 percent in spring and from 67.36±1.14 to 56.43±1.15 percent in summer.

Our findings are in close agreement with those of other workers (Bhalde *et al*., 1991; Datta *et al*., 1991 and Nath *et al*., 1991). Whareas Prabhu and Bhattacharya (1951) reported higher live sperm percent in Indian buffalo bull semen.

4.4.4 Post-thaw hypo-osmotic swelling test

Mean hypo-osmotic positive sperm was 43.87±1.04%. It ranged from 25 to 56% in spring season. Bulls did not differ significantly ($p<0.05$) in spring (Table no 6).

Mean hypo-osmotic positive sperm in summer was 41.85±1.32%. It ranged from 22 to 60%. Bulls differed significantly ($p<0.05$) to each other in summer season (Table no 6).

Our findings are slightly lower than the findings of Pant *et al*. (2002) and Pratap *et al*. (2000). In the present study, in all semen samples examined, some of the swollen spermatozoa were found to show non-progressive motility characterized by varying intensity of a flickering of the midpiece. This is in

agreement with an earlier report for bovine spermatozoa (Revell and Mrode, 1994).

Frozen thawed spermatozoa may represent a different situation as compared to the fresh non frozen spermatozoa, due to membrane alterations that could take place during freezing, cryostorage and thawing procedures (Saacke, 1961; Breddmann and Foote, 1969). Frozen-thawed specimens are subjected to membrane alterations during these procedures as evidenced by decreased and altered motion characteristics and decreased ability to penetrate cervical mucus. The analysis of variance between bulls in summer was significant which might be attributed to variation in sensitivity of individual bull seasonal changes.

4.4.5 Post-thaw cervical mucus penetration test

Mean penetration distance was 21.66±0.64 (range 11 to 30) and 17.59±0.64 (range 10 to 28) cm in spring and summer respectively. Bulls differed significantly ($p<0.05$) to each other in spring and summer and also between seasons (Table no 6).

The great reduction in the ability of spermatozoa to penetrate cervical mucus following cervical mucus following freezing is in agreement with the results for human spermatozoa (Urry *et al*., 1983) and bull semen (Matousek *et al*., 1989). A significant ($p<0.05$) difference between bulls and between season was observed which may be attributed to difference in various post-thaw cytomorphological characteristics which are known to affect penetration distance (Kumar and Devnathan, 1996 a)

4.4.6 Post-thaw percent abnormal sperm

Mean abnormal sperm was 18.11±0.50 (range 11 to 26), 19.17±0.86 (range 10 to 28) percent in spring and summer season respectively.

Bulls differ significantly ($p<0.05$) to each other. There was non significant ($p<0.05$) difference in sperm abnormalities in post thawed frozen semen samples between season (Table no 6 & fig no. 6 to 9).

The present findings on frozen semen are in accordance with that of other workers (Shetti *et al*., 1981; Hazarika *et al*., 1989 and Nath *et al*., 1991). The variation in the incidence of sperm abnormalities by different agroclimatic conditions (Saxena and Tripathi, 1985) and techniques employed (Rao and Rao, 1978).On the basis of present findings it can be stated that freezing of the breeding Murrah bulls semen had no significant effect on increase in the incidence of sperm abnormalities.

4.4.7 Post-thaw acrosomal integrity:

Mean acrosomal damage was 18.96±0.59 percent and ranged from 8 to 29 %. Bulls did not differ significantly to each other in spring (Table 6 & fig no. 10).

Mean acrosomal damage was 22.19±0.67 percent and ranged from 11 to 34% in summer season. Bulls differed significantly ($p<0.05$) to each other in summer season, significant difference was also observed between seasons (Table 6).

The acrosome which occupies upper most portion of the spermatozoa, takes part in the process of fertilization thus it is imperative to pay more attention on it. The apical ridge of the acrosome is likely to be damaged during the process of freezing causing loss of acrosomal enzymes (Church and Graves, 1979). According to Jones and Martin (1973) and Jones and Stewart (1979), the alteration in the acrosome takes place during their exposure to cold which causes considerable ultra-structural changes in acrosome leading to the disruption of

plasma membrane and outer acrosomal membrane resulting in the loss of acrosomal contents.

Post thaw acrosomal integrity varied significantly ($p<0.05$) between season. This might be attributed to sensitivity of individual bull spermatozoa to freezing stress.

4.5 Effect of duration of post-thaw incubation at (37^0 C) on post – thaw seminal characteristics:

4.5.1 Motility

Mean motility was 57.50±0.80 (range 50 to 70) 52.57±1.14 (range 30 to 70) percent after 15 min of incubation at 37^0 C in spring season and summer season respectively. (Table no. 7 & Fig no. 13, 14).

Mean motility was 52.50±0.80 (range 40 to 65) 48.50±1.18 (range 24 to 66) percent after 30 min of incubation at 37^0 C in spring and summer season respectively (Table no. 7 & Fig no. 13, 14).

Mean motility was 47.08±0.99 (range 30 to 65) 42.35±1.28 (range 12 to 60) percent after 45 min of incubation at 37^0 C in spring and summer season respectively (Table no. 7 & Fig no. 13, 14).

Mean motility was 38.83±0.85% (range 25 to 55) 30.66±1.15 (range 5 to 45) after 60 min of incubation at 37^0 C in spring and summer season respectively. There was significant ($p<0.05$) difference between bulls and between season (Table no. 7 & Fig no. 13, 14).

Our findings were comparable with Chinnaiya and Balakrishnan (1988) and Taraphder *et al*. (2001). Lower value was reported by Dhami *et al*. (1991).

4.5.2 Live sperm

Mean live sperm was 58.35±1.02 (range 36 to 74) 53.38±1.21 (range 23 to 77) percent after 15 min of incubation at 37^0 C in spring and summer respectively (Table no. 8 & Fig no. 13, 14).

Mean live sperm was 55.88±1.00 (range 34 to 71) 49.64±1.33 (range 18 to 75) percent after 30 min of incubation at 37^0 C in spring and summer respectively (Table no. 8 & Fig no. 13, 14).

Mean live sperm was 53.40±1.01 (range 30 to 68) 44.98±1.54 (range 10 to 73) percent in spring and summer season after 45 min of incubation at 37^0 C (Table no. 8 & Fig no. 13, 14).

Mean live sperm was 47.90±1.01 (range 23 to 65) 38.45±1.69 (range 4 to 67) percent in spring and summer season respectively after 60 min of incubation at 37^0 C. There was significant ($p<0.05$) difference between bulls and between season (Table no. 8 & Fig no. 13, 14).

Our findings are higher than that reported by Chinnaiya and Balakrishnan (1988).

4.5.3 Abnormal sperm

Mean abnormal sperm 18.70±0.52 (range 11 to 27) 19.98±0.91 (10 to 39) percent in spring and summer respectively after 15 min of incubation at 37^0 C (Table no. 9 & Fig no. 11 to 14).

Mean abnormal sperm 19.33±0.51 (range 12 to 27) 20.56±0.92 (range 11 to 39) percent in spring and summer respectively after 30 min of incubation at 37^0 C (Table no. 9 & Fig no. 11 to 14).

Mean abnormal sperm 20.01±0.54 (range 12 to 29) 21.14±0.91 (range 11 to 39) percent in spring and summer respectively after 45 min of incubation at 37^0 C (Table no. 9 & Fig no. 11 to 14).

Mean abnormal sperm 20.85±0.58 (range 13 to 31) 21.70±0.91 (range 12 to 40) percent in spring and summer respectively after 45 min of incubation at 37^0 C (Table no. 9 & Fig no. 11 to14).

Mean abnormal sperm does not increases significantly ($p<0.05$) between duration of incubation.

4.5.4 Acrosomal damage

Mean acrosomal damage was 19.88±0.62 (range 10 to 24) 23.15±0.71 (range 12 to 36) percent in spring and summer respectively after 15 min of incubation at 37^0 C. Bulls did not differ significantly ($p<0.05$) in spring (Table no. 10 & Fig no. 13, 14).

Mean acrosomal damage was 20.41±1.53 (range 11 to 26) 24.08±0.73 (range 13 to 38) percent in spring and summer respectively after 30 min of incubation at 37^0 C. Bulls did not differ significantly ($p<0.05$) in spring (Table no. 10 & Fig no. 13, 14).

Mean acrosomal damage was 21.05±0.63 (range 13 to 29) 24.92±0.75 (range 14 to 39) percent in spring and summer respectively after 45 min of incubation at 37^0 C. Bulls did not differ significantly ($p<0.05$) in spring (Table no. 10 & Fig no. 13, 14).

Mean acrosomal damage was 21.66±0.65 (range 15 to 23) 26.31±0.74 (range 17 to 43) percent in spring and summer respectively after 60 min of incubation at 37^0 C. Bulls did not differ significantly ($p<0.05$) in spring (Table no. 10 & Fig no. 13, 14).

Significant ($p<0.05$) difference between bulls in summer may be due to heat stress or individual variation. There was significant ($p<0.05$) difference between season (Table no. 10 & Fig no. 13, 14).

Chinnaiya and Balakrishnan (1988) reported higher acrosomal damage then our findings after one hour of incubation.

4.5.5 Hypo-osmotic swelling test

Mean hypo-osmotic swelled sperm was 5.93±0.22 (range 2 to 09) 5.89±0.33 (range 2 to 14) percent in spring and summer seasons respectively after 15 min of incubation at 37^0 C. Bulls did not differed significantly ($p<0.05$) to each other in spring season (Table no. 11 & Fig. No.13, 14).

Mean hypo-osmotic swelled sperm was 13.26±0.35 (range 6 to 18) 12.31±0.53 (range 5 to 21) percent in spring and summer seasons respectively after 30 min of incubation at 37^0 C. Bulls differed significantly ($p<0.05$) to each other in spring and summer season (Table no. 11 & Fig. No.13, 14).

Mean hypo-osmotic swelled sperm was 24.56±0.62 (range 11 to 34) 22.19±0.81 (range 11 to 39) percent in spring and summer seasons respectively after 45 min of incubation at 37^0 C. Bulls did not differed significantly ($p<0.05$) to each other in spring season (Table no. 11 & Fig. No.13, 14).

Mean hypo-osmotic swelled sperm was 40.31±1.05 (range 19 to 53) 38.61±1.32 (range 20 to 58) percent in spring and summer seasons respectively after 60 min of incubation at 37^0 C.

Hypo-osmotic swelled sperm increases continuously after incubation at 37^0 C till 60 min. in the present study (Table No. 11 & Fig. No.13, 14).

4.5.6 Cervical mucus penetration

Mean cervical mucus penetration distance was 9.33±0.32 (range 2 to 8) (range 2 to 7) millimeter after 15 min of incubation at 37^0 C (Table no. 12 & Fig no.13, 14).

Mean cervical mucus penetration distance was 4.58±0.19 (range 4 to 14) 7.91±0.33 (range 4 to 14) millimeter after 30 min of incubation at 37^0 C (Table no. 12 & Fig no.13, 14).

Mean cervical mucus penetration distance was 14.50±0.43 (range 7 to 21) 12.00±0.47 (range 7 to 21) millimeter after 30 min of incubation at 37^0 C (Table no. 12 & Fig no.13, 14).

Mean cervical mucus penetration distance was 20.40±0.63 (range 10 to 29) 16.61±0.64 (range 9 to 27) millimeter after 60 min of incubation at 37^0 C (Table no. 12 & Fig no.13, 14).

There was significant ($P<0.05$) difference between bulls in both season but not between seasons.

4.6. Effect of level of LN_2 and duration of storage on post-thaw seminal characteristics:

4.6.1 Level ¾ of straw

Motility

Mean motility was 59.83±0.68 (range 50 to 70) 54.23±0.76 (range 25 to 70) in spring and summer season respectively after 24 hours of storage (Table no. 13 & Fig no. 15, 16).

Mean motility was 55.50±0.74 (range 45 to 70) 49.03±1.25 (range 25 to 65) in spring and summer season respectively after 48 hours of storage (Table no. 13 & Fig no. 15, 16).

Mean motility was 50.58±0.86 (range 35 to 65) 45.17±1.18 (range 20 to 55) percent in spring and summer season respectively after 72 hours of storage (Table no. 13 & Fig no. 15, 16).

Mean motility was 44.58±1.03 (range 25 to 60) 39.91±1.15 (range 15 to 55) percent in spring and summer season respectively after 96 hours of storage (Table no. 13 & Fig no. 15, 16).

Mean motility was 39.95±1.05 (range 20 to 60) 33.01±1.12 (range 7 to 50) percent in spring and summer season respectively after 120 hours of storage.

There was significant ($p<0.05$) difference between duration of storage in both season (Table no. 13). Motility decrease at ¾ level of LN_2 in both season as duration increases.

Hypo-osmotic positive sperm

Mean hypo-osmotic swollen sperm percent was 41.80±1.08 (range 20 to 55) 37.25±0.87 (range 17 to 57) in spring and summer season respectively after 24 hours of storage (Table no.13 & Fig no.17, 18).

Mean hypo-osmotic swollen sperm percent was 38.90±1.10 (range 17 to 53) 34.03±0.86 (range 15 to 50) in spring and summer season respectively after 48 hours of storage (Table no.13 & Fig no.17, 18).

Mean hypo-osmotic swollen sperm percent was 36.11±1.08 (range 16 to 51) 30.17±0.82 (range 14 to 48) in spring and summer season respectively after 72 hours of storage (Table no.13 & Fig no.17, 18).

Mean hypo-osmotic swollen sperm percent was 32.65±1.08 (range 13 to 47) 27.12±0.27 (range 11 to 40) in spring and summer season respectively after 96 hours of storage There was significant ($p<0.05$) difference between duration (72 hrs to 96 hrs) of storage in both season. (Table no.13 & Fig no. 17, 18).

Mean hypo-osmotic swollen sperm percent was 28.76±1.06 (range 7 to 41) 24.20±0.45 (range 5 to 36) in spring and summer season respectively after 120 hours of storage. There was significant ($p<0.05$) difference between duration (96 hrs to 120 hrs) of storage in both season (Table no.13 & Fig no. 17, 18).

Abnormal sperm

Mean abnormal sperm percent was 18.41±0.49 (range 12 to 26) 19.21±0.12 (range 7 to 30) in spring and summer season respectively after 24 hours of storage (Table no.14 & Fig no.19, 20).

Mean abnormal sperm percent was 18.73±0.50 (range 12 to 27) 20.12±0.54 (range 8 to 31) in spring and summer season respectively after 48 hours of storage (Table no.14 & Fig no.19, 20).

Mean abnormal sperm percent was 19.26±0.50 (range 12 to 27) 20.08±1.24 (range 9 to 32) in spring and summer season respectively after 72 hours of storage (Table no.14 & Fig no.19, 20).

Mean abnormal sperm percent was 19.71±0.51 (range 13 to 28) 20.15±0.89 (range 9 to 33) in spring and summer season respectively after 96 hours of storage (Table no.14 & Fig no.19, 20).

Mean abnormal sperm percent was 20.11±0.51 (range 13 to 29) 20.45±1.20 (range 9 to 32) in spring and summer season respectively after 120 hours of storage (Table no.14 & Fig no.19, 20).

Abnormal sperm does not increases significantly ($p<0.05$) during duration of storage at ¾ level of LN_2 in both season (Table no. 14).

Acrosomal damage

Mean acrosomal damage sperm percent was 19.80±4.67 (range 9 to 30) 24.27±1.29 (range 12 to 37) in spring and summer season respectively after 24 hours of storage (Table no. 14 & Fig no. 21, 22).

Mean acrosomal damage sperm percent was 19.95±0.60 (range 9 to 31) 25.26±1.13 (range 12 to 39) in spring and summer season respectively after 48 hours of storage (Table no. 14 & Fig no. 21, 22).

Mean acrosomal damage sperm percent was 20.63±0.61 (range 9 to 32) 28.25±2.05 (range 13 to 40) in spring and summer season respectively after 72 hours of storage (Table no. 14 & Fig no. 21, 22).

Mean acrosomal damage sperm percent was 21.40±0.61 (range 10 to 33) 28.76±1.08 (range 13 to 41) in spring and summer season respectively after 96 hours of storage (Table no. 14 & Fig no. 21, 22).

Mean acrosomal damage sperm percent was 22.28±0.61 (range 11 to 35) 31.08±1.12 (range 14 to 42) in spring and summer season respectively after 120 hours of storage. There was significant ($p<0.05$) difference in summer between 96 to 120 hours of storage (Table no. 14 & Fig no. 21, 22).

Cervical mucus penetration

Mean cervical mucus penetration distance was 20.41±0.62 (range 7 to 29) 17.37±1.21 (range 9 to 24) millimeter in spring and summer season after 24 hours of storage (Table no 15 & Fig no. 23, 24).

Mean cervical mucus penetration distance was 18.86±0.60 (range 6 to 28) 17.01±1.08 (range 8 to 22) millimeter in spring and summer season after 48 hours of storage (Table no 15 & Fig no. 23, 24).

Mean cervical mucus penetration distance was 16.66±0.59 (range 6 to 26) 15.21±0.78 (range 4 to 21) millimeter in spring and summer season after 72 hours of storage (Table no 15 & Fig no. 23, 24).

Mean cervical mucus penetration distance was 14.48±0.59 (range 4 to 23) 13.78±0.45 (range 4 to 19) millimeter in spring and summer season after 96 hours of storage (Table no 15 & Fig no. 23, 24).

Mean cervical mucus penetration distance was 10.40±0.50 (range 2 to 18) 8.59±0.23 (range 1 to 16) millimeter in spring and summer season after 120 hours of storage. There was significant ($p<0.05$) difference in cervical mucus penetration distance in between 96 to 120 hours (Table no 15 & Fig no. 23, 24).

4.6.2 Level ½ of straw

Motility

Mean motility was 50.41±0.79 (range 40 to 65) 48.25±1.21 (range 20 to 70) in spring and summer season respectively after 24 hours of storage (Table no. 13 & Fig no. 15, 16).

Mean motility was 42.25±6.06 (range 30 to 55) 37.05±0.88 (range 15 to 60) in spring and summer season respectively after 48 hours of storage (Table no. 13 & Fig no. 15, 16).

Mean motility was 33.66±0.86 (range 20 to 50) 28.24±0.12 (range 10 to 55) in spring and summer season respectively after 72 hours of storage (Table no. 13 & Fig no. 15, 16).

Mean motility was 25.00±0.90 (range 10 to 40) 21.14±0.23 (range 10 to 50) in spring and summer season respectively after 96 hours of storage (Table no. 13 & Fig no. 15, 16).

Mean motility was 19.03±0.83 (range 4 to 35) 14.21±1.20 (range 5 to 30) in spring and summer season respectively after 120 hours of storage (Table no. 13 & Fig no. 15, 16).

There was significant ($p<0.05$) difference in motility between duration of storage in both season (Table no. 13). Motility decrease more rapidly as compare to ¾ to 1/2 level of LN_2 (Fig no. 15 &16).

Hypo-osmotic positive sperm

Mean hypo-osmotic swollen sperm percent was 38.60±0.96 (range 21 to 51) 31.21±0.51 (range 13 to 54) in spring and summer season respectively after 24 hours of storage (Table no.13 & Fig no.17, 18).

Mean hypo-osmotic swollen sperm percent was 31.90±0.84 (range 15 to 46) 26.24±0.62 (range 11 to 45) in spring and summer season respectively after 48 hours of storage (Table no.13 & Fig no.17, 18).

Mean hypo-osmotic swollen sperm percent was 25.56±0.75 (range 11 to 41) 20.12±1.07 (range 10 to 40) in spring and summer season respectively after 72 hours of storage (Table no.13 & Fig no.17, 18).

Mean hypo-osmotic swollen sperm percent was 19.16±0.71 (range 07 to 35) 12.84±1.04 (range 8 to 30) in spring and summer season respectively after 96 hours of storage (Table no.13 & Fig no.17, 18).

Mean hypo-osmotic swollen sperm percent was 12.85±0.70 (range 03 to 25) 09.12±0.24 (range 4 to 20) in spring and summer season respectively after 120 hours of storage (Table no.13 & Fig no.17, 18).

There was significant ($p<0.05$) difference in hypo-osmotic positive sperm percentage between duration of storage in both season (Table no. 13).

Abnormal sperm

Mean abnormal sperm percent was 19.33±2.05 (range 13 to 27) 20.22±0.47 (range 12 to 30) in spring and summer season respectively after 24 hours of storage (Table no.14 & Fig no.19, 20).

Mean abnormal sperm percent was 20.05±0.48 (range 14 to 27) 21.24±1.04 (range 13 to 31) in spring and summer season respectively after 48 hours of storage (Table no.14 & Fig no.19, 20).

Mean abnormal sperm percent was 19.37±0.27 (range 14 to 27) 21.57±1.09 (range 12 to 30) in spring and summer season respectively after 72 hours of storage (Table no.14 & Fig no.19, 20).

Mean abnormal sperm percent was 20.38±0.50 (range 13 to 29) 21.34±0.63 (range 13 to 32) in spring and summer season respectively after 96 hours of storage (Table no.14 & Fig no.19, 20).

Mean abnormal sperm percent was 20.78±0.51 (range 13 to 28) 22.23±0.95 (range 13 to 33) in spring and summer season respectively after 120 hours of storage (Table no.14 & Fig no.19, 20).

Abnormal sperm does not increases significantly ($p<0.05$) during duration of storage at 1/2 level of LN_2 in both season (Table no. 14).

Acrosomal damage

Mean acrosomal damage sperm percent was 21.03±0.61 (range 12 to 32) 27.25±1.34 (range 16 to 43) in spring and summer season respectively after 24 hours of storage (Table no. 14 & Fig no. 21, 22).

Mean acrosomal damage sperm percent was 24.03±0.61 (range 14 to 35) 30.12±1.07 (range 16 to 44) in spring and summer season respectively after 48 hours of storage (Table no. 14 & Fig no. 21, 22).

Mean acrosomal damage sperm percent was 27.10±0.66 (range 15 to 38) 32.25±1.28 (range 20 to 43) in spring and summer season respectively after 72 hours of storage (Table no. 14 & Fig no. 21, 22).

Mean acrosomal damage sperm percent was 30.10±0.74 (range 16 to 42) 33.25±1.21 (range 20 to 45) in spring and summer season respectively after 96 hours of storage (Table no. 14 & Fig no. 21, 22).

Mean acrosomal damage sperm percent was 33.13±0.82 (range 17 to 47) 37.05±1.24 (range 21 to 52) in spring and summer season respectively after 120 hours of storage (Table no. 14 & Fig no. 21, 22).

Cervical mucus penetration

Mean cervical mucus penetration distance was 18.96±0.61 (range 10 to 27) 16.04±1.08 (range 8 to 26) millimeter in spring and summer season after 24 hours of storage (Table no 15 & Fig no. 23, 24).

Mean cervical mucus penetration distance was 15.53±0.51 (range 8 to 23) 13.25±0.52 (range 6 to 21) millimeter in spring and summer season after 48 hours of storage (Table no 15 & Fig no. 23, 24).

Mean cervical mucus penetration distance was 12.15±0.44 (range 5 to 19) 10.24±1.04 (range 3 to 15) millimeter in spring and summer season after 72 hours of storage (Table no 15 & Fig no. 23, 24).

Mean cervical mucus penetration distance was 9.11±0.38 (range 3 to 14) 7.12±0.15 (range 3 to 12) millimeter in spring and summer season after 96 hours of storage (Table no 15 & Fig no. 23, 24).

Mean cervical mucus penetration distance was 6.20±0.33 (range 2 to 14) 5.01±0.44 (range 1 to 10) millimeter in spring and summer season after 120 hours of storage (Table no 15 & Fig no. 23, 24).

4.6.3 Level 1/4 of straw

Motility

Mean motility was 47.78±5.85 (range 35 to 60) 41.25±0.78 (range 25 to 55) in spring and summer season respectively after 24 hours of storage (Table no. 13 & Fig no. 15, 16).

Mean motility was 35.75±0.74 (range 25 to 50) 29.27±1.23 (range 20 to 50) in spring and summer season respectively after 48 hours of storage (Table no. 13 & Fig no. 15, 16).

Mean motility was 26.16±0.77 (range 15 to 40) 21.15±0.74 (range 10 to 40) in spring and summer season respectively after 72 hours of storage (Table no. 13 & Fig no. 15, 16).

Mean motility was 16.66±0.74 (range 5 to 30) 10.02±0.25 (range 5 to 20) in spring and summer season respectively after 96 hours of storage (Table no. 13 & Fig no. 15, 16).

Mean motility was 8.43±0.53 (range 0 to 20) 4.24±1.21 (range 0 to 15) in spring and summer season respectively after 120 hours of storage (Table no. 13 & Fig no. 15, 16).

There was significant ($p<0.05$) difference in motility between duration of storage in both season (Table no. 13). Motility decrease more rapidly as compare to ¾ and 1/2 level of LN_2 to ¼ level (Fig no. 15, 16).

Hypo-osmotic positive sperm

Mean hypo-osmotic swollen sperm percent was 31.68±0.67 (range 23 to 44) 26.24±1.04 (range 19 to 38) in spring and summer season respectively after 24 hours of storage (Table no.13 & Fig no.17, 18).

Mean hypo-osmotic swollen sperm percent was 24.55±0.61 (range 15 to 36) 19.85±1.20 (range 14 to 30) in spring and summer season respectively after 48 hours of storage (Table no.13 & Fig no.17, 18).

Mean hypo-osmotic swollen sperm percent was 17.90±0.63 (range 07 to 31) 14.50±0.17 (range 8 to 25) in spring and summer season respectively after 72 hours of storage (Table no.13 & Fig no.17, 18).

Mean hypo-osmotic swollen sperm percent was 10.55±0.58 (range 01 to 22) 7.02±1.08 (range 3 to 18) in spring and summer season respectively after 96 hours of storage (Table no.13 & Fig no.17, 18).

Mean hypo-osmotic swollen sperm percent was 4.26±0.41 (range 00 to 12) 02.01±1.23 (range 0 to 7) in spring and summer season respectively after 120 hours of storage (Table no.13 & Fig no.17, 18).

There was significant ($p<0.05$) difference in hypo-osmotic positive sperm percentage between each observation of storage in both season at ¼ level of LN_2 (Table no. 13).

Abnormal sperm

Mean abnormal sperm percent was 18.85±0.48 (range 12 to 27) 21.27±1.14 (range 13 to 31) in spring and summer season respectively after 24 hours of storage (Table no.14 & Fig no.19, 20).

Mean abnormal sperm percent was 19.03±0.49 (range 12 to 27) 22.24±1.21 (range 13 to 30) in spring and summer season respectively after 48 hours of storage (Table no.14 & Fig no.19, 20).

Mean abnormal sperm percent was 19.51±0.49 (range 13 to 28) 22.86±0.60 (range 14 to 32) in spring and summer season respectively after 72 hours of storage (Table no.14 & Fig no.19, 20).

Mean abnormal sperm percent was 20.01±0.49 (range 13 to 28) 22.97±1.24 (range 14 to 33) in spring and summer season respectively after 96 hours of storage (Table no.14 & Fig no.19, 20).

Mean abnormal sperm percent was 20.56±0.50 (range 13 to 29) 22.18±0.59 (range 14 to 33) in spring and summer season respectively after 120 hours of storage (Table no.14 & Fig no.19, 20).

Abnormal sperm does not increases significantly ($p<0.05$) during duration of storage at 1/4 level of LN_2 in both season (Table no. 14).

Acrosomal damage

Mean acrosomal damage sperm percent was 23.30±0.61 (range 13 to 36) 32.02±1.09 (range 18 to 43) in spring and summer season respectively after 24 hours of storage (Table no. 14 & Fig no. 21, 22).

Mean acrosomal damage sperm percent was 27.20±0.61 (range 18 to 40) 33.12±1.23 (range 22 to 47) in spring and summer season respectively after 48 hours of storage (Table no. 14 & Fig no. 21, 22).

Mean acrosomal damage sperm percent was 31.23±0.64 (range 21 to 45) 35±26±1.51 (range 29 to 55) in spring and summer season respectively after 72 hours of storage (Table no. 14 & Fig no. 21, 22).

Mean acrosomal damage sperm percent was 35.16±0.65 (range 25 to 49) 38.05±1.05 (range 29 to 55) in spring and summer season respectively after 96 hours of storage (Table no. 14 & Fig no. 21, 22).

Mean acrosomal damage sperm percent was 39.05±0.70 (range 28 to 53) 45.07±1.27 (range 31 to 58) in spring and summer season respectively after 120 hours of storage (Table no. 14 & Fig no. 21, 22).

Significant ($p<0.05$) difference was observed during each observation at ¼ level of LN_2 in both season.

Cervical mucus penetration

Mean cervical mucus penetration distance was 17.81±0.55 (range 9 to 25) 14.28±1.21 (range 8 to 24) millimeter in spring and summer season after 24 hours of storage (Table no 15 & Fig no. 23, 24).

Mean cervical mucus penetration distance was 13.31±0.45 (range 5 to 19) 12.07±1.24 (range 4 to 17) millimeter in spring and summer season after 48 hours of storage (Table no 15 & Fig no. 23, 24).

Mean cervical mucus penetration distance was 9.08±0.36 (range 2 to 14) 7.02±1.31 (range 1 to 15) millimeter in spring and summer season after 72 hours of storage. Significant ($p<0.05$) difference between (72 to 96 hours) was observed in both season (Table no 15 & Fig no. 23, 24).

Mean cervical mucus penetration distance was 5.25±0.30 (range 0 to 10) 5.25±0.30 (range 1 to 8) millimeter in spring and summer season after 96 hours of storage. Significant ($p<0.05$) difference between (96 to 120 hours) was observed in both season (Table no 15 & Fig no. 23, 24).

Mean cervical mucus penetration distance was 2.08±0.16 (range 0 to 6) 1.54±0.78 (range 0 to 3) millimeter in spring and summer season after 120 hours of storage (Table no 15 & Fig no. 23, 24).

CHAPTER – V

SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH WORK

The study entitled “Seminal characteristic and freezability of Murrah bull semen during spring and summer seasons” was carried out at Central Semen Station Anjora, Durg (Chhattisgarh).

The study was conducted on six Murrah bull semen. The objectives of the study were to study the correlation of testicular biometry and reaction time with semen production, physio-morphological characteristics and freezability of Murrah bull semen during spring and summer seasons, the effect of duration of post thaw incubation on different seminal characteristics and the effect of level of liquid nitrogen in container and duration of incubation on seminal characteristics.

Immediately after collection, the semen samples were evaluated for volume, colour, concentration, mass motility, initial progressive motility, percent abnormal sperm, percent intact acrosome. A small quantity of semen was used for cervical mucus penetration test (CMPT) and hypo-osmotic swelling test (HOST).

After evaluating the physio-morphological characteristics, semen was extended in the Tris dilutor. The semen was diluted so as to maintain 20 million sperm/ml of diluted semen. The dilutors contained 6.4% glycerol and 20% of egg yolk. The extended semen after filling in French mini straws was kept in cooling cabinet. A combined cooling cum equilibration period of 4 hours was given for cooling the straws up to 4^0 C. After equilibration prefreeze motility was assessed. The precooled straws were kept in LN_2 vapors and once the temperature of straws reached to -110^0 to -120^0 C, they were plunged in LN_2 and stored. After 24 hours of storage in LN_2 the straws were taken out and thawed at 37^0 C for 35 sec. Motility, live sperm and acrosomal integrity of thawed semen were assayed at this stage.

Scrotal circumference differed significantly ($P<0.05$) between bulls in both season and it had positive correlation with ejaculate volume, concentration and live sperm. Scrotal volume differed significantly ($P<0.05$) between bulls and there was non significant ($P<0.05$) difference between season. Scrotal volume had also

positive correlation with ejaculate volume, concentration and live sperm percent. Reaction time did not differ significantly ($P<0.05$) between season but it differs significantly ($P<0.05$) between bulls. It had negative correlation with ejaculate volume, concentration, live sperm percent. Ejaculate volume did not differed significantly ($P<0.05$) between season but differed significantly ($P<0.05$) between bulls in both season. Semen colour differed significantly ($P<0.05$) between bulls in summer. Sperm concentration and initial progressive motility differed significantly ($P<0.05$) between bulls in both season. Initial progressive motility differed significantly ($P<0.05$) between season. Hypo-osmotic swelled sperm and differ significantly ($P<0.05$) between bulls in summer and did not differed significantly between season. Cervical mucus penetration distance differ significantly ($P<0.05$) between bulls and differed significantly ($P<0.01$) between season. Live sperm percent differed significantly ($P<0.05$) between bulls in both season and also between season. Acrosomal damage differed significantly ($P<0.05$) between bulls in both season but not between season. Abnormal sperm percent did not differed significantly ($P<0.05$) between season. Prefreeze motility, post thaw motility, live sperm percent, hypo-osmotic swelled spermatozoa (%), cervical mucus penetration distance (mm), and acrosomal abnormality differed significantly ($P<0.05$) between season in the frozen post-thaw semen. Abnormal sperm percent did not differed significantly ($P<0.05$) between seasons.

Motility decreases from 64.83±0.68 to 38.83±0.85% in spring and 58.64±1.34 to 30.66±1.15% in summer season similarly live percent decreases from 61.45±0.99 to 47.90±1.01% in spring and 56.43±1.15 to 38.45±1.69% in summer season after one hour of post-thaw incubation at 37^0 C. Abnormal sperm increases from 18.11±0.50 to 20.85±0.58% and 19.17±0.86 to 21.70±0.91 in spring and summer season. Acrosomal damage increases from 18.96±0.59 to 21.66±0.65% in spring and 22.19±0.67 to 26.31±0.84 in summer season one hour of post-thaw incubation at 37^0 C. Hypo-osmotic positive sperm percent increases up to 40.31±1.05 and 38.61±1.32% in spring and summer season after one hour of post-thaw incubation at 37^0 C. Similarly Cervical mucus penetration distance reached 20.40±0.63 and 16.61±0.64 mm in spring and summer season after one hour of incubation at 37^0 C.

Motility decreases continuously in ¾ level to ½. It decreases significantly ($P<0.05$) on every 24 hrs to 120 hrs. Hypo-osmotic swelling percent decreases significantly at ½ and ¼ level LN_2 of straw. Abnormal sperm percent does not increases significantly ($P<0.05$) at different level of LN_2 while acrosomal damage increases significantly at ¼ level of LN_2. Cervical mucus penetration distance decreases significantly ($P<0.05$) at ¼ level of LN_2 in the container.

Conclusions

Based on the findings of the present study it could be concluded that

1.) Scrotal circumference and volume are highly correlated to the ejaculate volume, concentration and live sperm.
2.) The semen quality of Murrah bulls is better in spring than summer season.
3.) Certain Murrah bulls are more sensitive to the agro climatic changes so there was individual variation.
4.) Level of LN_2 in container should always be full. Because semen quality decreases vary rapidly as LN_2 level goes down.

Suggestions for Future Research Work

1) Before selection of a Murrah bull for semen freezing subsequently used for A.I. programme, a complete screening should be done on various seminal characteristics and it's freezability under the existing agro-climatic condition.
2) Grading of semen quality should be correlated with their exact field fertility trials.
3) Study may be conducted in large number of Murrah bulls and round the year.
4) Ultra structural changes in spermatozoa during freezing, storage and post-thaw may be studied using electron microscope.

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SEMINAL CHARACTERISTICS AND FREEZABILITY OF MURRAH BULL SEMEN DURING SPRING AND SUMMER SEASONS

By
SUBODH KUMAR

ABSTRACT

The experiment was undertaken to estimate the correlation of testicular biometry and reaction time with semen production in Murrah bulls, to study the physio-morphological characteristics and freezability of Murrah bull semen during spring and summer, see the effect of duration of incubation period on post-thaw seminal characteristics and see the effect of level of liquid nitrogen in container and duration of storage on post thaw seminal characteristics.

Six Murrah bulls age between 5 to 7 years was experimental animals. The bulls were used routinely for semen collection and they were maintained under identical feeding and management regimes during the entire course of study.

Scrotal circumference and scrotal volume differed significantly ($p<0.05$) between bulls in both season. Scrotal circumference and scrotal volume had positive correlation with ejaculate volume, concentration and live sperm. Reaction time does not differed significantly ($p<0.05$) between season. It had positive correlation with abnormal sperm Ejaculate volume did not differed significantly ($p<0.05$) between season. Semen colour differed significantly ($p<0.05$) between bulls in summer but not between season. Initial progressive motility differed significantly ($p<0.05$) between season. Sperm concentration did not differed significantly ($p<0.05$) between season. Hypo-osmotic swelled sperm differ significantly ($p<0.05$) between bulls in summer and did not differ significantly between season. Cervical mucus penetration distance differed significantly ($p<0.05$) between bulls and also differed significantly ($p<0.01$) between season. Live sperm percent differed significantly ($p<0.05$) between bulls in both season and also between season. Acrosomal damage differed significantly ($p<0.05$) between bulls in both season but not between season. Abnormal sperm percent did not differed significantly ($p<0.05$) between season.

Post thaw motility, live sperm percent, hypo-osmotic swelled spermatozoa, cervical mucus penetration distance, acrosomal abnormality differed significantly ($p<0.05$) between seasons. Abnormal sperm percent did not differed significantly ($p<0.05$) between seasons.

Motility decreases from 64.83±0.68 to 38.83±0.85% and 58.64±1.34 to 30.66±1.15% in spring and summer season respectively. Live percent decreases from 61.45±0.99 to 47.90±1.01% and 56.43±1.15 to 38.45±1.69% in spring and summer season respectively. Abnormal sperm increases from 18.11±0.50 to 20.85±0.58% and 19.17±0.86 to 21.70±0.91 in spring and summer season respectively. Acrosomal damage increases from 18.96±0.59 to 21.66±0.65% and 22.19±0.67 to 26.31±0.84 spring and summer respectively. Hypo-osmotic positive sperm percent increases up to 40.31±1.05 and 38.61±1.32% in spring and summer respectively. Similarly Cervical mucus penetration distance reached 20.40±0.63 and 16.61±0.64 mm in spring and summer season after 1 hr. of post-thaw incubation at 37^0 C.

Motility decreases continuously in ¾ level to ½.level of LN_2. It decreases significantly ($p<0.05$) on every 24 hrs upto 120 hrs. Hypo-osmotic swelling percent decreases significantly at ½ and ¼ levels LN_2 of straw. Abnormal sperm percent does not increases significantly ($p<0.05$) at different level of LN_2 while acrosomal damage increases significantly at ¼ level of LN_2. Cervical mucus penetration distance decreases significantly ($p<0.05$) during different observation interval at ¼ level of LN_2 in the container and also ½ level of LN_2 in the container.

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SEMINAL CHARACTERISTICS AND FREEZABILITY OF MURRAH BULL SEMEN DURING SPRING AND SUMMER SEASONS

M.V.Sc. THESIS ABSTRACT

by

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ROLL NO.8641 ID NO. UG/ VETY/2001/74

DEPARTMENT OF ANIMAL REPRODUCTION GYNAECOLOGY AND OBSTETRICS

COLLEGE OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY
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2008

DEPARTMENT OF ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS
COLLEGE OF VETERINARY SCINENCE, IGKV, ANJORA, DURG

APPLICATION FOR SUBMISSION OF M.V.Sc. THESIS

(Acad. Reg. for Master Degree Programme:16.06; Appendix VII of Manual of Style for Thesis Writing)

1. Name of the Student	:	Subodh Kumar
2. ID Number	:	UG/VETY/DURG/2001/74
3. College	:	College of Veterinary Science & AH, Anjora, Durg
4. Date of First Registration	:	January 05, 2007
5. Whether regular or Inservice Student	:	Regular
6. Title of the Thesis	:	SEMINAL CHARACTERISTICS AND FREEZABILITY OF MURRAH BULL SEMEN DURING SPRING AND SUMMER SEASONS
7. Major Subject	:	ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS.
8. Date of Approval of Advisory Committee	:	05/ 07/ 2008
9. Whether Course Credit completed or not	:	Completed
10 The OGPA of Course Credits	:	8.55
11. Ratio of Major and Minor Course	:	29:13
12. Expected Date of Submission Thesis	:	November 10, 2008
13. If Submitted after Due Date whether permitted	:	To be submitted within the due date

Major Advisor : **Dr. R.P. Tiwari**
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And Obstetrics

Whether Panel of Examiners submitted or not : Submitted

HEAD OF THE DEPARTMENT

Fig no. 1 Murrah bulls during semen collection

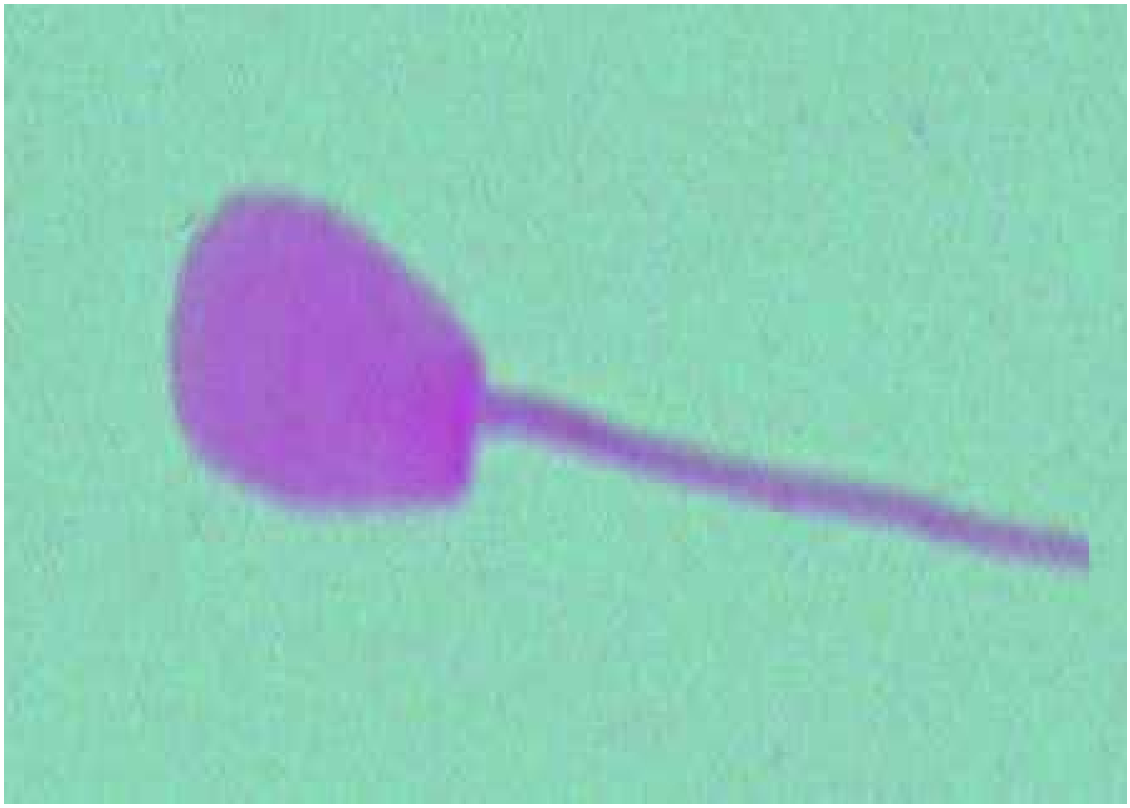

Fig no. 2 Spermatozoa with abaxial attachment

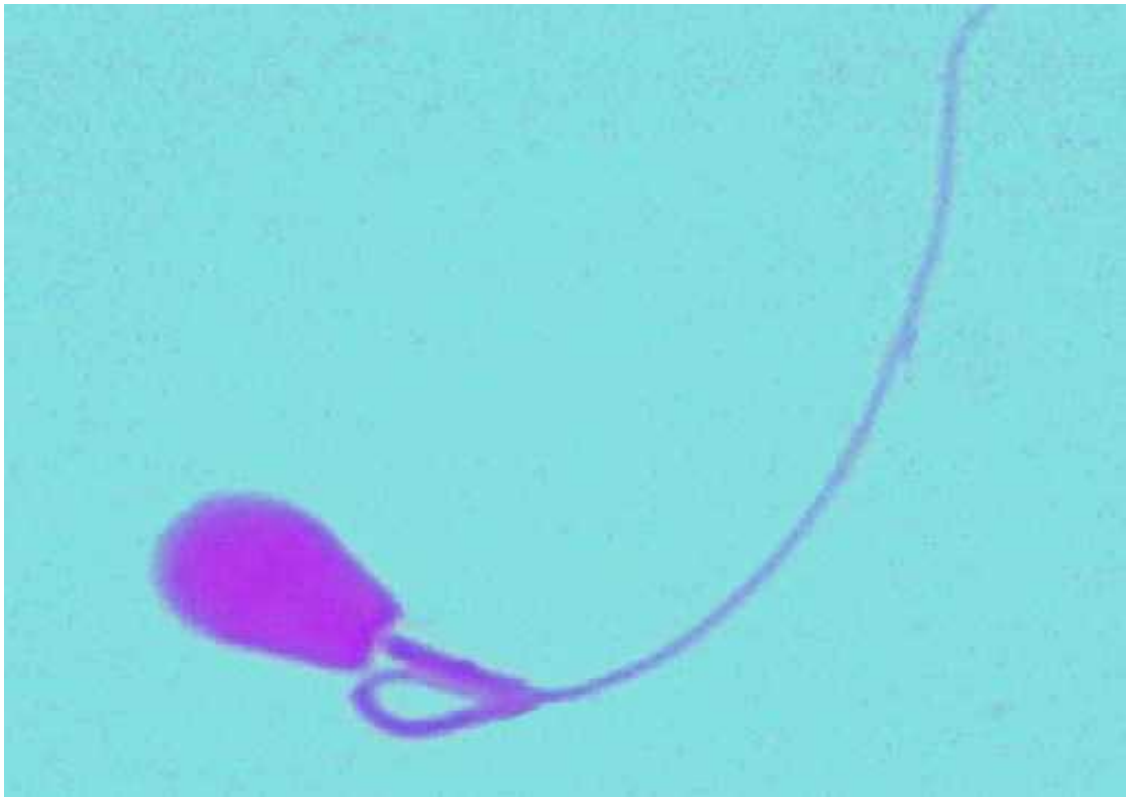

Fig no. 3 Spermatozoa with coiled midpiece

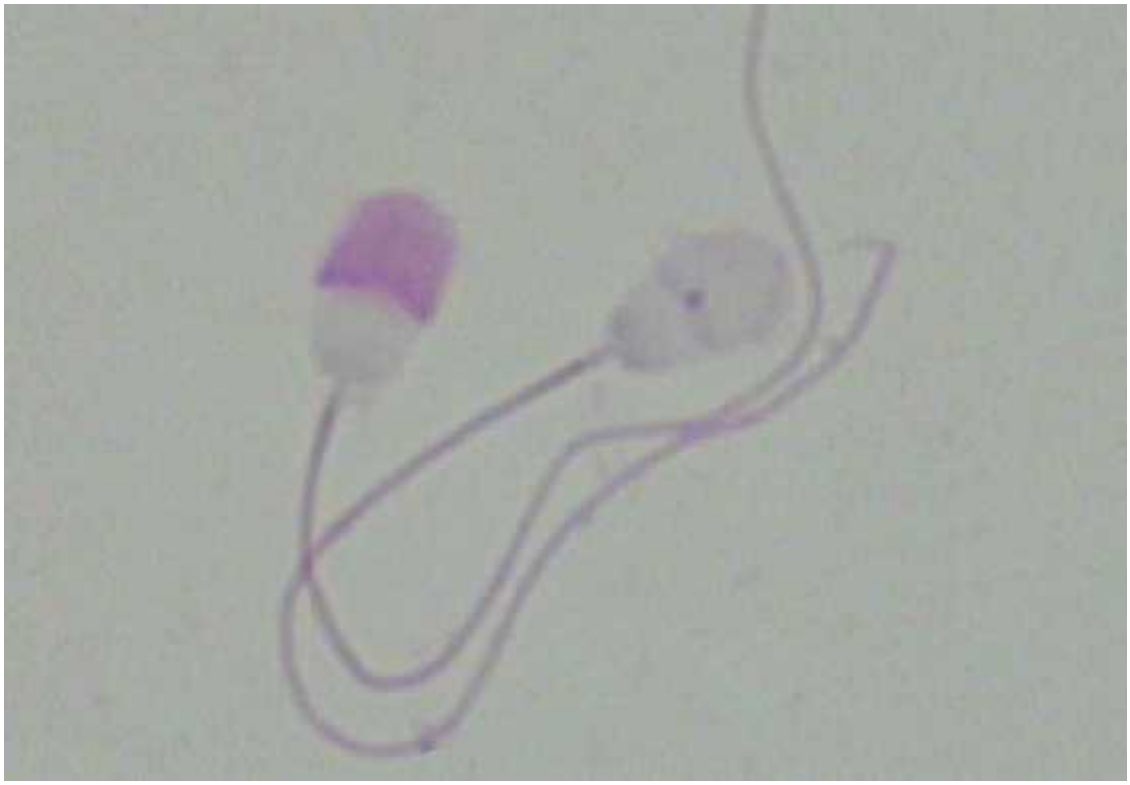

Fig no. 4 Normal spermatozoa (Left one) and complete cap separation (Right one)

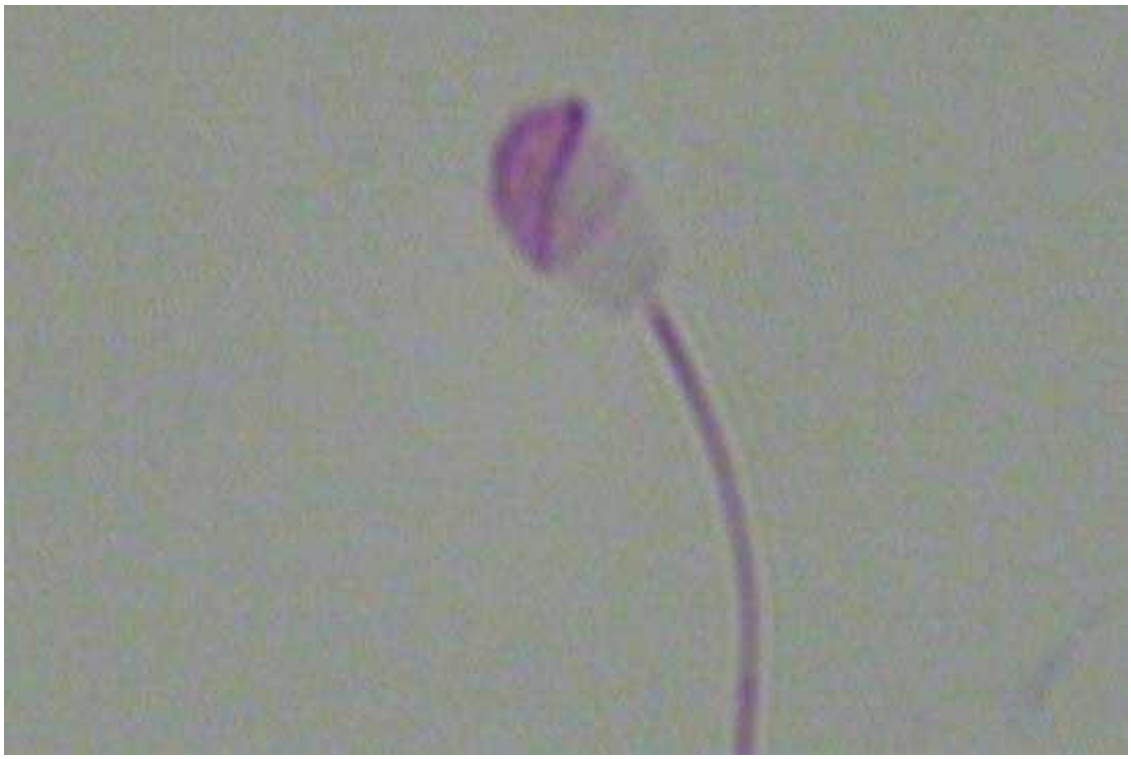

Fig no. 5 Spermatozoa having incomplete cap separation

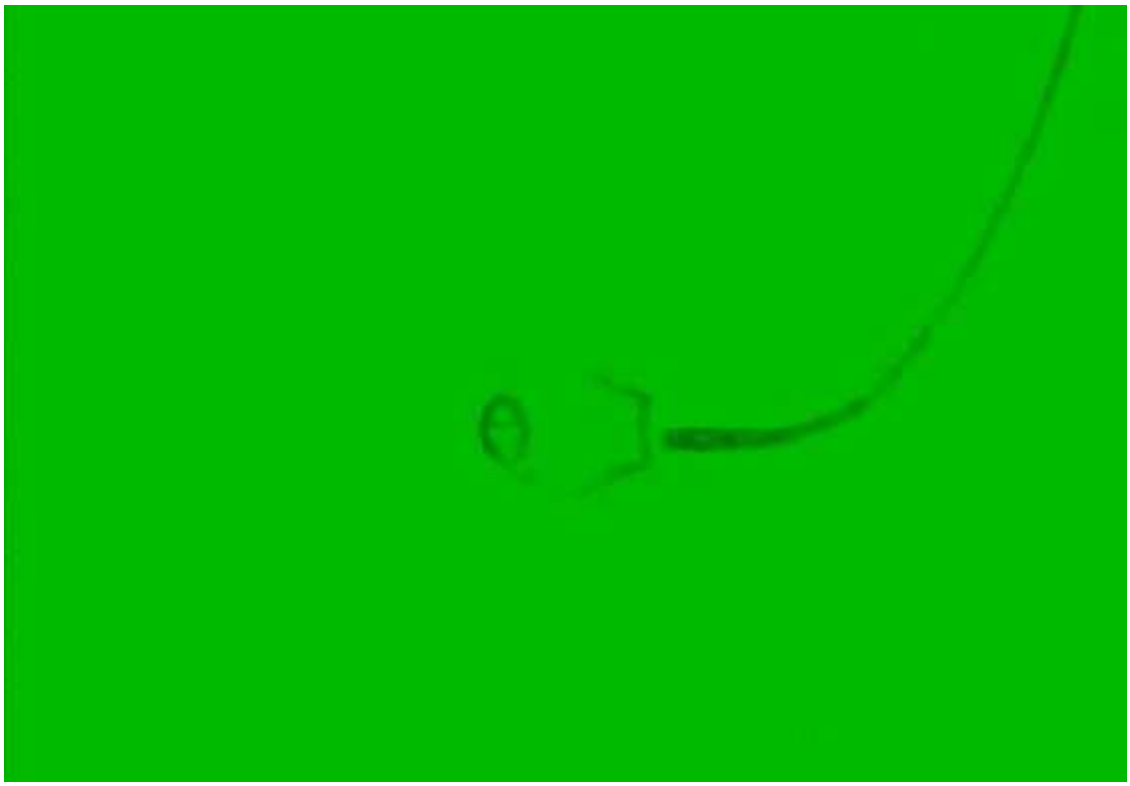

Fig no. 6 Spermatozoa with knobbed acrosome

Fig no. 7 Spermatozoa with dag defect

Fig no. 8 Spermatozoa with double head

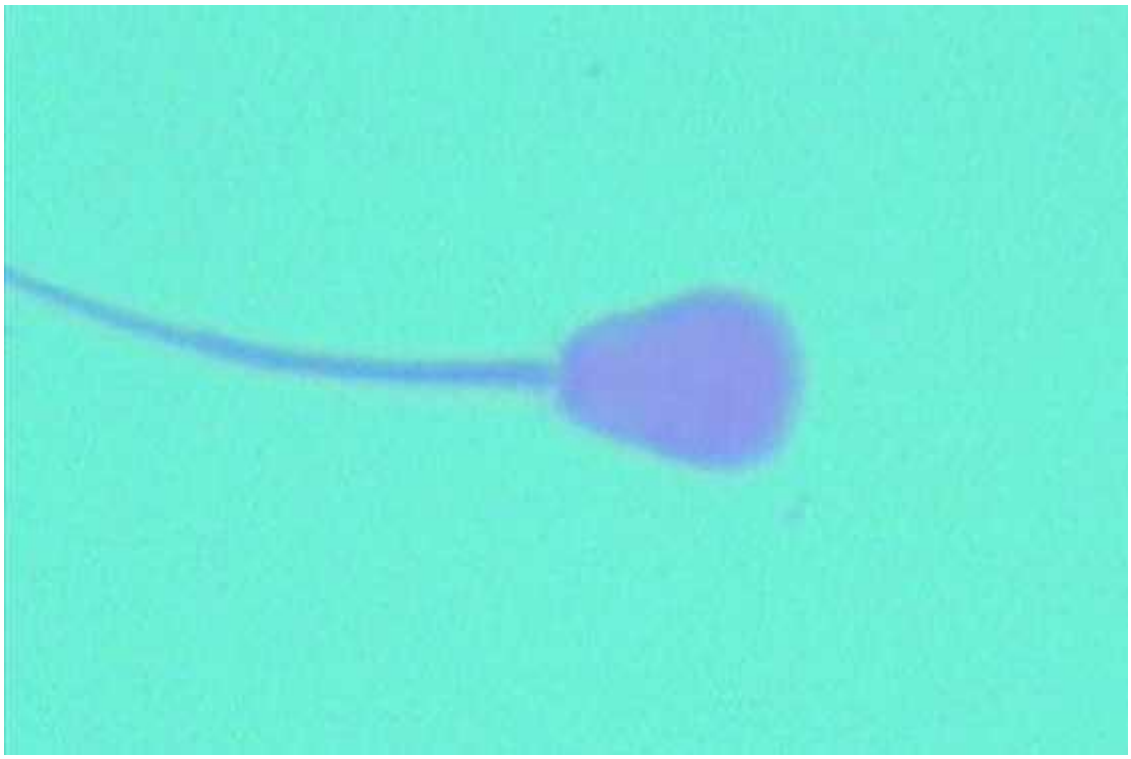

Fig no. 9 Spermatozoa with pear shape head

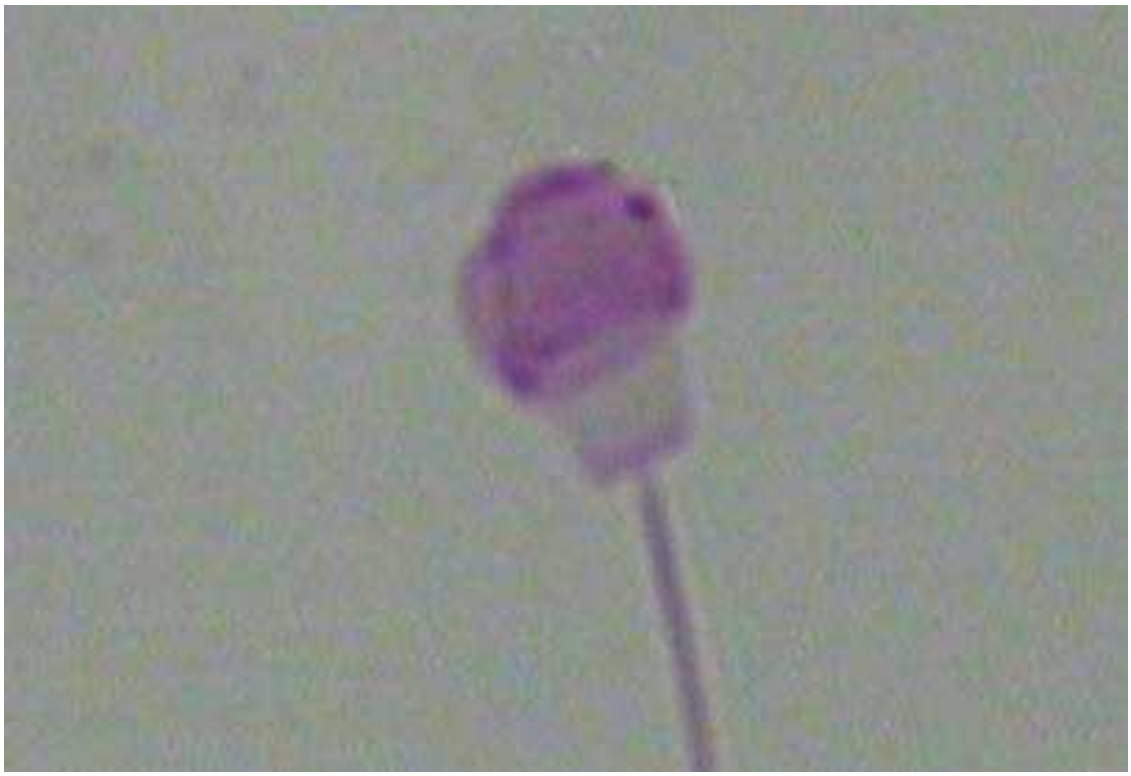

Fig no. 10 Spermatozoa with swollen acrosome

Fig no. 11 Spermatozoa having thick mid piece

Fig no.12. Spermatozoa with loose head attachment

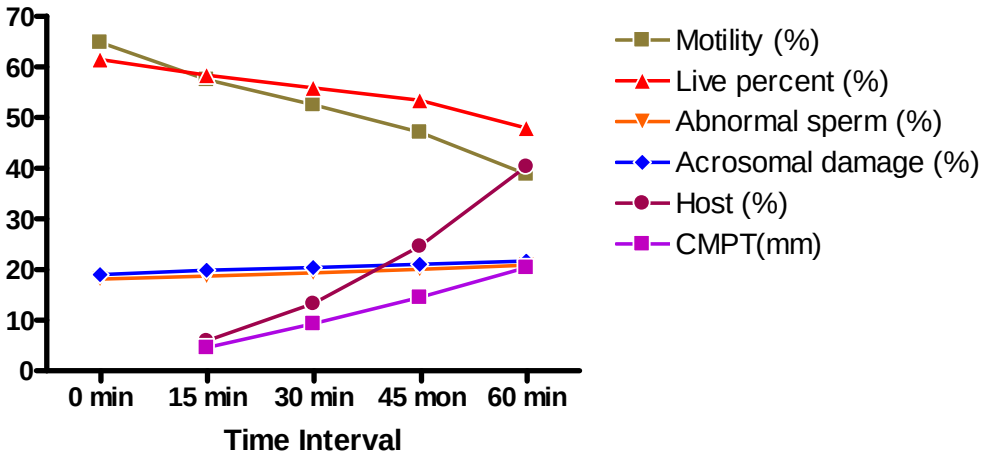

Fig no. 13 Effect of duration of incubation at 37^0 C on motility (%), live percent (%), abnormal sperm (%), acrosomal damage (%), HOST (%) and CMPT (mm) in spring.

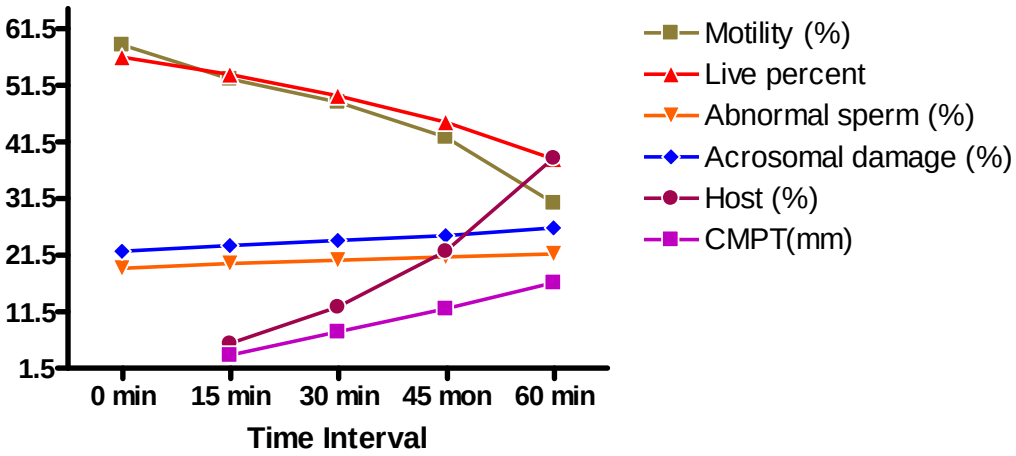

Fig no. 14 Effect of duration of incubation at 37^0 C on motility (%), live percent (%), abnormal sperm (%), acrosomal damage (%), HOST (%) and CMPT (mm) in summer.

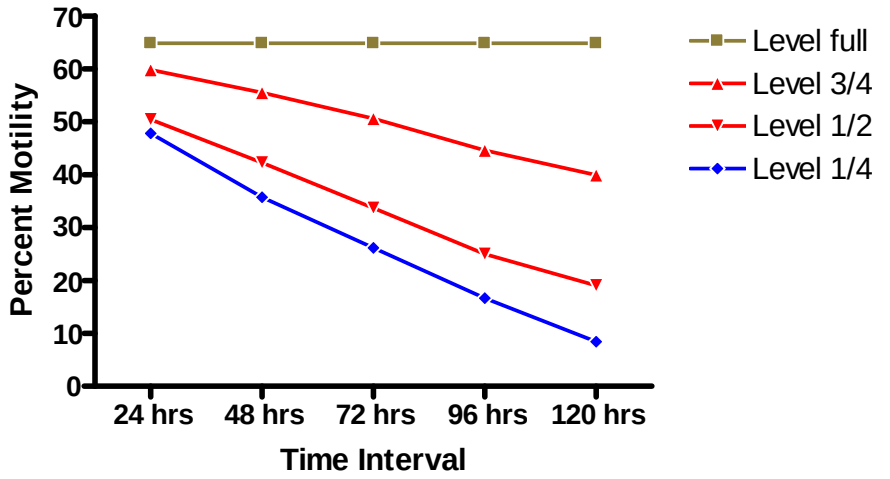

Fig no.15 Effect of level of LN_2 and duration of storage on post-thaw motility of semen of Murrah buffalo bulls in spring.

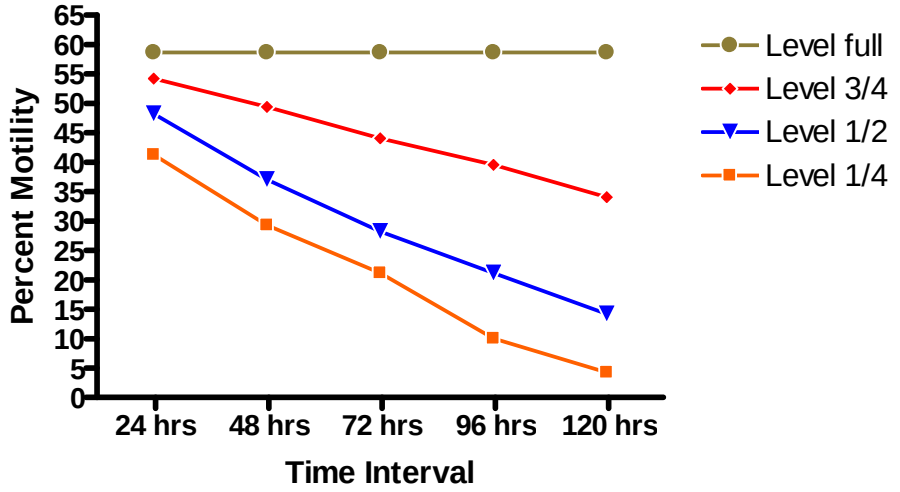

Fig no. 16 Effect of level of LN_2 and duration of storage on post-thaw motility of semen of Murrah buffalo bulls in summer.

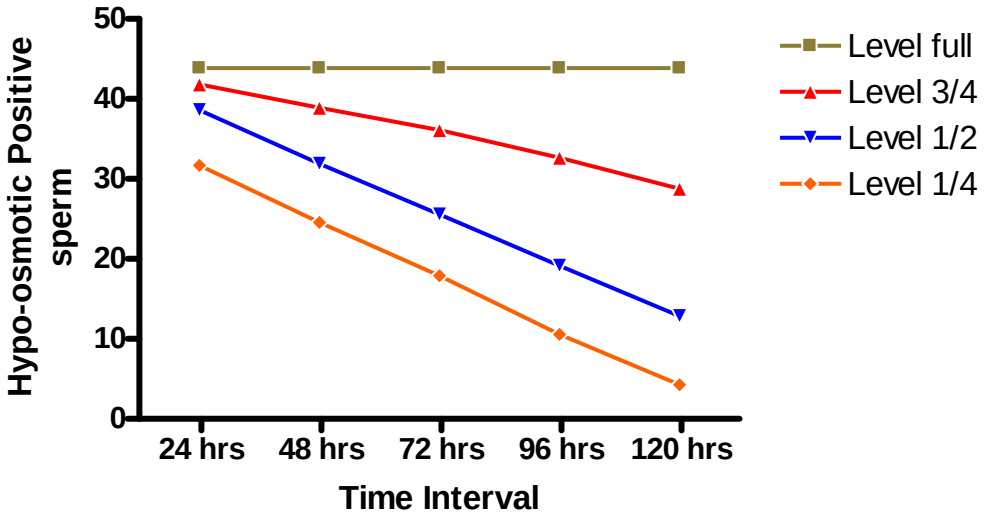

Fig no.17 Effect of level of LN2 and duration of storage on Hypo-osmotic positive sperm of Murrah buffalo bulls in spring.

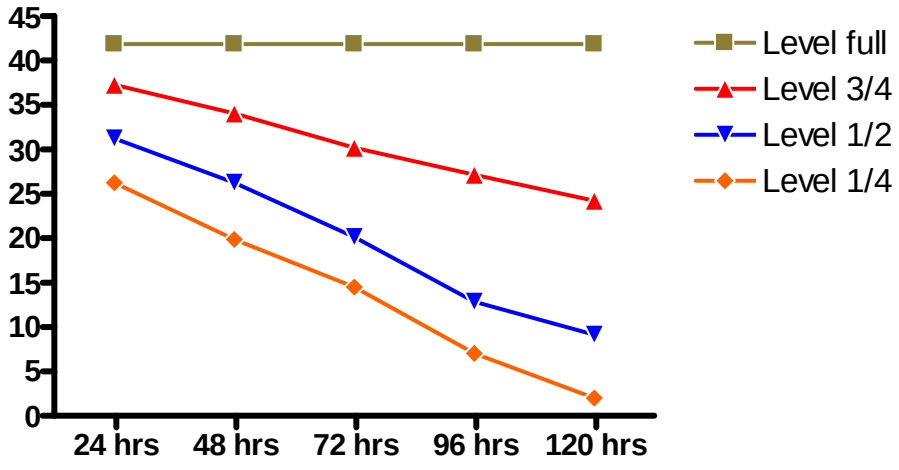

Fig no.18 Effect of level of LN_2 and duration of storage on Hypo-osmotic positive sperm of Murrah buffalo bulls in summer.

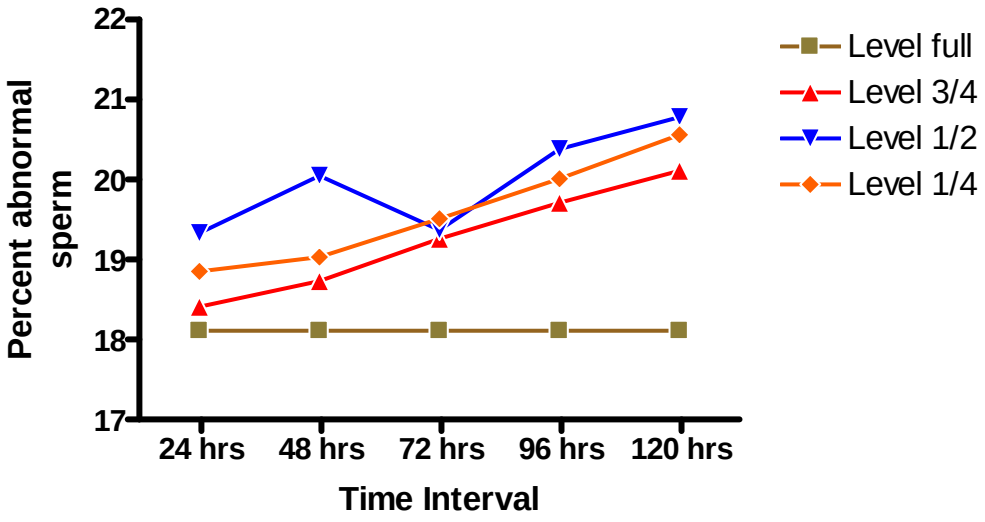

Fig no. 19 Effect of level of LN_2 and duration of storage on abnormal sperm percent of Murrah buffalo bulls in spring.

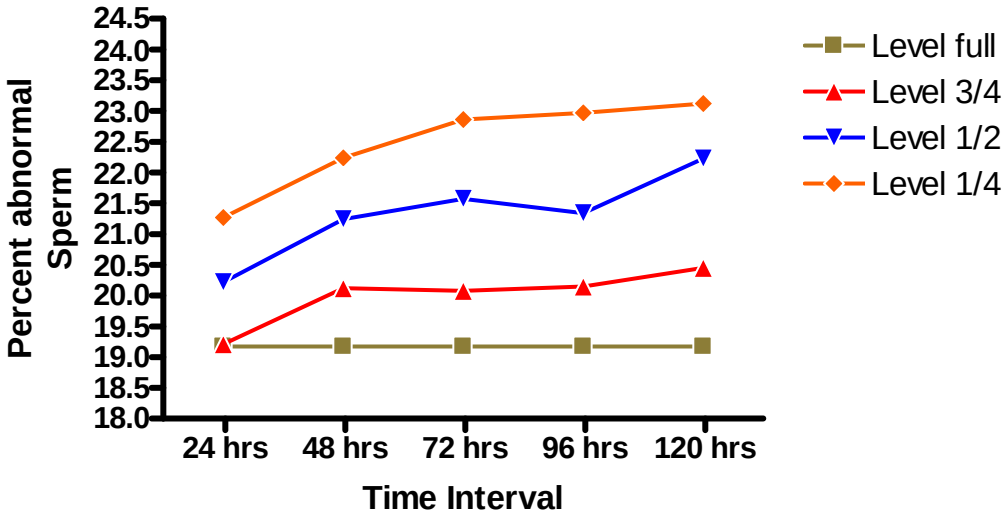

Fig no. 20 Effect of level of LN_2 and duration of storage on abnormal sperm percent of Murrah buffalo bulls in summer.

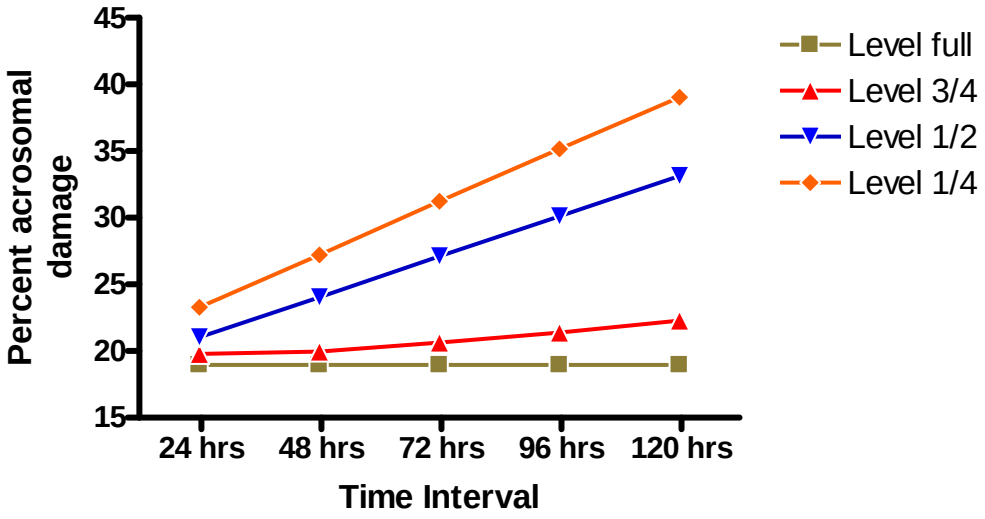

Fig no. 21 Effect of level of LN_2 and duration of storage on acrosomal damage percent of Murrah buffalo bulls in spring.

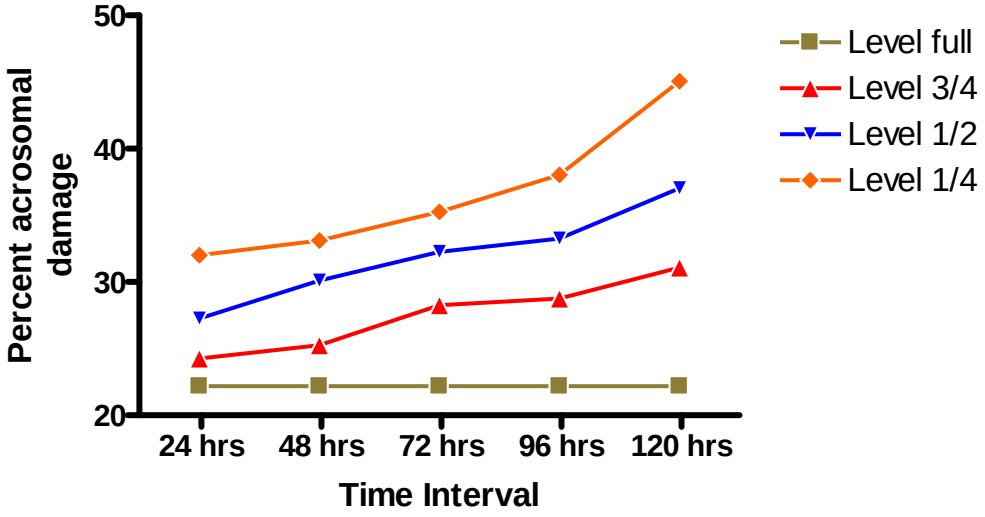

Fig no. 22 Effect of level of LN_2 and duration of storage on acrosomal damage percent of Murrah buffalo bulls in summer.

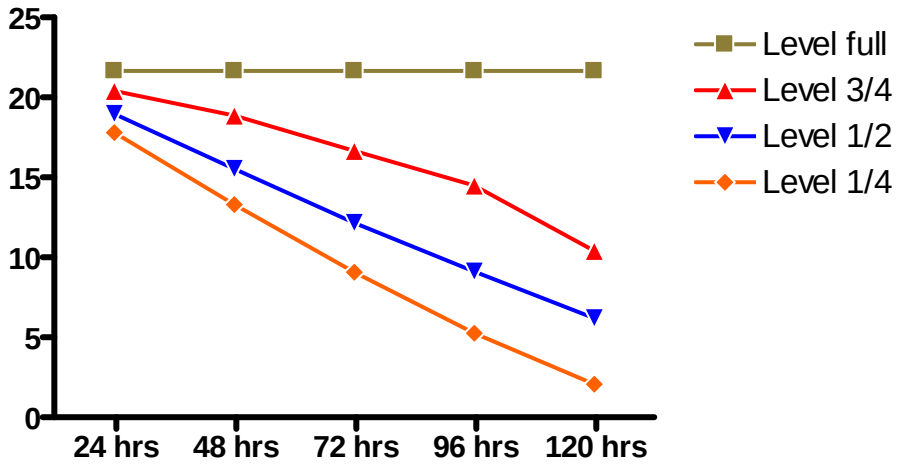

Fig no. 23 Effect of level of LN_2 and duration of storage on Cervical mucus penetration distance Murrah buffalo bulls in spring.

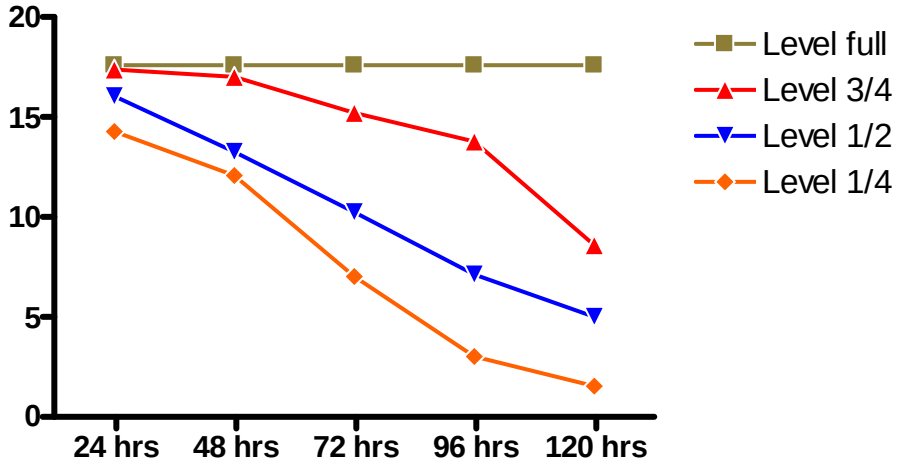

Fig no. 24 Effect of level of LN_2 and duration of storage on Cervical mucus penetration distance Murrah buffalo bulls in summer.

Table No. 2 Scrotal Circumference and Scrotal Volume of Murrah breeding bulls in Spring & Summer

Bulls	Scrotal Circumference (cm)		Scrotal Volume (ml)		Scrotal Volume (cm^3)	
	Spring	Summer	Spring	Summer	Spring	Summer
Bull No 429	35.96^{e}±0.11	36.11^{e}±0.12	811.00^{e}±7.66	821.00^{d}±11.63	789.39^{e}±0.66	785.28^{e}±0.88
Bull No 525	33.99^{d}±0.11	33.92^{d}±0.07	692.00^{f}±6.28	687.30^{c}±0.70	660.08^{d}±0.34	658.08^{d}±0.44
Bull No 560	29.67^{a}±0.13	29.94^{a}±0.12	638.00^{c}±4.66	667.20^{c}±24.13	623.38^{c}±0.57	624.98^{c}±0.69
Bull No 555	36.92^{f}±0.08	37.06^{f}±0.07	902.00^{a}±4.66	908.80^{e}±1.43	880.22^{f}±0.55	880.84^{f}±0.87
Bull No 570	31.01^{b}±0.07	31.05^{b}±0.08	476.00^{a}±3.05	474.85^{a}±1.82	460.35^{a}±0.69	461.57^{a}±0.65
Bull No 559	32.91^{c}±0.06	33.04^{c}±0.05	537.00^{b}±3.00	532.10^{b}±1.20	524.86^{b}±0.70	523.72^{b}±0.45
Overall Mean	33.41±0.33	33.65±0.34	676.00±19.30	682.17±18.91	656.38±18.77	665.96±18.72

Superscripts are read columns wise for comparison of mean. Different superscripts differed significantly (P< 0.05)

Table No. 3 Correlation coefficient of various parameter during Spring & Summer

Parameters	Ejaculate volume (ml)		Concentration (Million/ml)		Live sperm (%)		Abnormal sperm (%)	
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer
Scrotal Circumference (cm)	0.182	0.161	0.210	0.082	0.217	0.195	-0.136	-0.130
Scrotal Volume (ml)	0.441*	0.300	0.143	0.121	0.211	0.181	-0.131	-0.100
Scrotal Volume (cm^3)	0.438*	0.282	0.118	0.970	0.270	0.151	-0.137	-0.109
Reaction Time (Sec)	-0.208	-0.226	-0.649**	-0.588**	-0.543**	-0.421**	0.479**	0.532**

* Significant p<0.05
** Significant p<0.01

Table No. 4 Reaction time in Spring and Summer season

	Reaction time	
Bull No	**Spring**	**Summer**
Bull no 429	94.30^{a}±7.50	116.60^{ab}±16.08*
Bull no 525	123.10^{a}±13.64	143.80^{ab}±10.98*
Bull no 560	112.40^{a}±15.58	95.60^{a}±8.65
Bull no 555	109.40^{a}±16.21	106.30^{a}±15.69
Bull no 570	123.80^{a}±15.80	166.28^{b}±43.23*
Bull no 559	137.20^{b}±21.17	126.50^{ab}±11.26
Overall Mean	**116.70±6.31**	**123.71±7.53**

Superscripts are read columns wise for comparison of mean. Different superscripts differed significantly ($P< 0.05$)

* Significant $p<0.05$ between season

Table No 5 Seminal characteristics of fresh semen of Murrah bulls during spring and summer season

Parameters	Season	Bull No 429	Bull No 525	Bull No 560	Bull No 555	Bull No 570	Bull No 559	Overall Mean
Ejaculate Volume (ml)	Spring	4.09^{bc}±0.47	4.08^{bc}±0.46	4.69^{c}±0.35	5.28^{c}±1.04	2.76^{ab}±0.26	2.10^{a}±0.18	3.83±0.25
	Summer	4.70^{bc} ± 0.29	3.70^{b} ± 0.52	5.31^{c} ± 0.60	4.07^{bc} ± 0.53	2.21^{a} ± 0.54	3.54^{ab} ± 0.30	4.02±0.22
Mass Activity (0-5 Scale)	Spring	3.80^{a}±0.24	3.20^{a}±0.32	3.50^{a}±0.16	3.30^{a}±0.30	3.20^{a}±0.24	3.40^{a}±0.30	3.40±0.10
	Summer	3.50^{b} ± 0.40	2.10^{a} ± 0.27	3.50^{b} ± 0.26	3.40^{b} ± 0.30	2.85^{ab}±0.34	3.80^{b}±0.32	3.21±0.14
Semen Colour (0-2 Scale)	Spring	1.80^{a}±0.13	1.50^{a}±0.16	1.70^{a}±0.15	1.60^{a}±0.16	1.50^{a}±0.16	1.50^{a}±0.22	1.60±0.06
	Summer	1.60^{b} ± 0.16	0.60^{a} ± 0.16	1.50^{b} ± 0.16	1.40^{b} ± 0.22	1.28^{b}±0.28	1.70^{b}±0.15	1.35±0.08
Concentration (Million/ml)	Spring	1674.72^{b}±136.24	1162.25^{ab}±198.53	1245.38^{ab}±141.5	1407.73^{ab}±164.06	1091.54^{a}±121.81	1664.79^{b}±246.45	1374.40±74.11
	Summer	1671.41^{b} ± 207.20	731.90^{a} ± 101.09	1511.78^{b} ± 182.58	1614.20^{b} ± 252.9	1243.84^{ab}±190.73	1693.72^{b} ± 192.57	1419.94±88.9
Initial Progressive Motility (%)	Spring	88.50^{c}±1.06	82.50^{ab}±1.34	81.50^{a}±1.29	84.00^{ab}±1.79	81.00^{a}±1.45	86.00^{bc}±1.45	83.91±0.65
	Summer	85.00^{b} ± 1.29	77.50^{b} ± 4.72	76.50^{b} ± 3.07	75.00^{b} ± 4.14	63.57^{a} ± 4.04	81.00^{b} ± 2.56	77.10±1.57*
Hypo-osmotic positive sperm (%)	Spring	55.80^{a}±1.81	53.00^{a}±2.55	51.50^{a}±1.80	50.35^{a}±3.10	50.05^{a}±2.34	55.40^{a}±2.10	52.68±0.95
	Summer	55.75^{b} ± 2.93	39.20^{a} ± 2.25	49.3^{b} ± 2.41	51.55^{b} ± 3.82	49.71^{b} ± 2.98	55.80^{b} ±1.55	50.24±1.31
Cervical mucus penetration (mm)	Spring	28.90^{a}±1.43	26.20^{a}±1.56	26.80^{a}±1.41	26.60^{a}±1.77	24.10^{a}±1.27	24.50^{a}±2.10	26.18±0.66
	Summer	26.90^{b} ± 1.74	19.60^{a} ± 1.12	19.91^{a} ± 1.41	23.50^{ab} ± 2.07	19.71^{a} ± 0.97	23.40^{ab} ± 1.24	22.29±0.69*
Live sperm percent	Spring	76.00^{ab}±2.53	73.50^{ab}±3.33	76.30^{ab}±2.33	75.40^{ab}±2.20	72.50^{a}±1.87	81.55^{b}±2.76	75.87±1.12
	Summer	78.50^{c} ± 2.16	65.00^{ab} ± 1.54	65.50^{a} ± 1.43	62.80^{ab} ± 3.31	61.71^{a} ± 2.88	69.00^{b} ± 1.47	67.36±1.14*
Abnormal sperm percent	Spring	12.70^{a}±0.98	13.00^{a}±1.34	13.80^{a}±1.29	14.10^{a}±1.64	16.60^{a}±1.27	12.80^{a}±1.23	13.83±0.54
	Summer	10.80^{a} ±1.24	19.70^{b}±1.71	11.80^{a} ±1.00	13.40^{a} ± 1.41	14.85^{a}±1.78	12.90^{a}±1.06	13.85±0.66
Acrosomal abnormalities percent	Spring	13.60^{a}±1.00	17.30^{b}±1.35	13.60^{a}±0.76	14.70^{ab}±1.49	18.00^{b}±1.45	12.60^{a}±0.93	14.80±0.54
	Summer	14.90^{ab} ± 0.78	17.70^{bc}±1.24	15.50^{abc} ±0.67	15.80^{abc} ± 1.49	18.71^{c}±1.37	13.00^{a}±0.81	15.78±0.48

Superscripts are read row wise for comparison of mean. Different superscripts differed significantly ($p < 0.05$)
* Differ significantly between season ($p<0.05$)

Table No. 7 Effect of duration of post-thaw incubation at 37^0 C on Motility in Spring and Summer

Bull No	Motility %									
	0 min		15 min		30 min		45 min		60 min	
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer
429	$68.50^{c} \pm 1.50$	$61.00^{b} \pm 1.24$	$62.00^{cd} \pm 1.33$	$59.20^{b} \pm 1.87$	$56.00^{c} \pm 1.45$	$55.20^{b} \pm 2.03$	$51.00^{c} \pm 1.79$	$52.00^{b} \pm 1.85$	$42.50^{c} \pm 1.34$	$34.40^{b} \pm 1.83$
525	$63.50^{ab} \pm 1.06$	$57.20^{ab} \pm 4.18$	$51.00^{a} \pm 0.66$	$49.50^{a} \pm 2.41$	$47.00^{a} \pm 1.33$	$45.30^{a} \pm 2.56$	$40.50^{a} \pm 1.89$	$43.00^{a} \pm 2.80$	$36.00^{ab} \pm 1.63$	$29.70^{a} \pm 3.55$
560	$63.00^{ab} \pm 1.10$	$59.10^{b} \pm 2.50$	$55.50^{bc} \pm 1.57$	$52.90^{ab} \pm 2.26$	$52.50^{bc} \pm 1.34$	$49.20^{ab} \pm 2.24$	$47.50^{bc} \pm 1.34$	$42.10^{a} \pm 2.32$	$41.50^{c} \pm 1.06$	$32.60^{a} \pm 2.22$
555	$69.00^{c} \pm 1.94$	$60.50^{b} \pm 4.04$	$64.50^{d} \pm 1.89$	$52.80^{ab} \pm 3.72$	$61.00^{d} \pm 1.24$	$48.10^{ab} \pm 3.90$	$57.50^{d} \pm 1.70$	$39.50^{a} \pm 4.35$	$48.00^{a} \pm 1.69$	$27.50^{a} \pm 3.54$
570	$59.50^{a} \pm 1.16$	$49.28^{a} \pm 4.29$	$53.00^{ab} \pm 1.10$	$45.85^{a} \pm 3.04$	$47.00^{a} \pm 1.52$	$42.00^{a} \pm 3.22$	$40.50^{a} \pm 1.38$	$37.28^{a} \pm 3.59$	$32.50^{a} \pm 1.53$	$26.85^{a} \pm 3.09$
559	$65.50^{bc} \pm 1.16$	$62.00^{c} \pm 2.49$	$59.00^{cd} \pm 0.66$	$53.20^{ab} \pm 2.02$	$51.50^{b} \pm 0.76$	$49.30^{ab} \pm 2.03$	$45.50^{b} \pm 1.16$	$38.70^{a} \pm 1.53$	$38.50^{bc} \pm 1.30$	$31.80^{a} \pm 2.11$
Overall Mean	**64.83 ± 0.68**	**58.64±1.34***	**57.50 ± 0.80**	**52.57±1.14***	**52.50±0.81**	**48.50±1.18***	**47.08±0.99**	**42.35±1.28***	**38.83±0.85**	**30.66±1.15***

Superscripts are read columns wise for comparison of mean. Different superscripts differed significantly ($p < 0.05$)
* Significant ($p<0.05$) between season

Table No. 8 Effect of duration of post-thaw incubation at 37^0 C on Live percent in Spring and Summer

Bull No	Live Percent									
	0 min		15 min		30 min		45 min		60 min	
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer
429	$62.00^{a} \pm 2.40$	$66.60^{c} \pm 2.04$	$59.30^{a} \pm 2.46$	$63.70^{c} \pm 2.21$	$56.30^{a} \pm 2.47$	$61.00^{b} \pm 2.54$	$53.90^{a} \pm 2.49$	$58.20^{c} \pm 2.94$	$48.80^{a} \pm 2.28$	$51.90^{c} \pm 3.37$
525	$59.90^{a} \pm 2.70$	$52.70^{a} \pm 1.89$	$57.20^{a} \pm 2.72$	$49.90^{ab} \pm 2.06$	$55.40^{a} \pm 2.60$	$45.40^{a} \pm 2.40$	$53.50^{a} \pm 2.51$	$39.20^{a} \pm 3.14$	$49.20^{a} \pm 2.48$	$31.30^{a} \pm 3.84$
560	$62.00^{a} \pm 1.98$	$54.10^{ab} \pm 1.30$	$59.50^{a} \pm 2.14$	$50.90^{ab} \pm 1.53$	$56.70^{a} \pm 2.13$	$47.40^{a} \pm 1.81$	$54.30^{a} \pm 2.09$	$42.90^{ab} \pm 2.12$	$48.30^{a} \pm 2.06$	$37.20^{ab} \pm 2.35$
555	$60.90^{a} \pm 2.13$	$52.00^{a} \pm 3.50$	$57.40^{a} \pm 2.14$	$49.00^{a} \pm 3.89$	$55.30^{a} \pm 2.15$	$44.70^{a} \pm 4.17$	$52.80^{a} \pm 2.17$	$38.70^{a} \pm 4.65$	$46.90^{a} \pm 2.13$	$30.10^{a} \pm 4.65$
570	$58.35^{a} \pm 2.11$	$51.57^{a} \pm 2.85$	$54.80^{a} \pm 2.22$	$48.57^{a} \pm 2.90$	$52.60^{a} \pm 2.22$	$44.70^{a} \pm 3.09$	$50.00^{a} \pm 2.31$	$40.40^{ab} \pm 2.91$	$43.90^{a} \pm 2.37$	$34.71^{b} \pm 4.11$
559	$65.60^{b} \pm 3.10$	$60.20^{bc} \pm 1.62$	$61.90^{b} \pm 3.17$	$56.80^{bc} \pm 1.61$	$59.00^{b} \pm 3.17$	$53.20^{ab} \pm 1.63$	$55.90^{b} \pm 3.32$	$49.10^{b} \pm 1.55$	$50.30^{b} \pm 3.45$	$44.40^{bc} \pm 1.49$
Overall Mean	**61.45±0.99**	**56.43±1.15***	**58.35±1.02**	**53.38±1.21***	**55.88±1.00**	**49.64±1.33***	**53.40±1.01**	**44.98±1.54***	**47.90±1.01**	**38.45±1.69***

Superscripts are read columns wise for comparison of mean. Different superscripts differed significantly ($p < 0.05$)
* Significant ($p<0.05$) between season

Table No. 9 Effect of duration of post-thaw incubation at 37^0 C on Abnormal Sperm (%) in Spring and Summer

Bulls	Abnormal Sperm%									
	0 min		15 min		30 min		45 min		60 min	
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer
Bull No 429	16.70^{ab}±1.13	15.30^{a}±1.47	17.00^{ab}±1.20	16.00^{a}±1.67	17.70^{ab}±1.22	16.40^{a}±1.63	18.30^{ab}±1.43	17.30^{a}±1.67	19.30^{ab}±1.53	17.40^{a}±1.63
Bull No 525	17.70^{ab}±1.27	26.80^{b}±2.23	18.30^{ab}±1.36	27.80^{b}±2.39	19.00^{ab}±1.29	28.60^{b}±2.43	19.60^{ab}±1.34	28.80^{b}±2.46	20.30^{ab}±1.51	29.60^{b}±2.43
Bull No 560	19.30^{ab}±1.13	17.60^{a}±1.27	20.30^{ab}±1.13	18.30^{a}±1.52	21.30^{b}±1.13	18.90^{a}±1.50	22.00^{b}±1.11	19.40^{a}±1.50	22.80^{b}±1.09	20.10^{a}±1.53
Bull No 555	20.10^{b}±1.32	19.20^{a}±2.32	20.70^{b}±1.33	19.70^{a}±2.31	21.30^{b}±1.40	20.20a±2.36	22.00^{b}±1.42	20.60^{a}±2.36	23.10^{b}±1.79	21.10^{a}±2.35
Bull No 570	19.30^{ab}±1.14	20.00^{a}±1.42	19.80^{ab}±1.01	21.00^{a}±1.73	20.20^{ab}±0.98	21.57^{ab}±1.87	21.00^{ab}±1.00	22.14^{a}±1.83	21.50^{ab}±1.00	22.85^{a}±1.80
Bull No 559	15.60^{a}±1.09	16.40^{a}±1.38	16.10^{a}±1.17	17.40^{a}±1.40	16.70^{a}±1.10	18.00^{a}±1.35	17.20^{a}±1.16	18.90^{a}±1.35	18.10^{a}±1.14	19.50^{a}±1.38
Overall Mean	**18.11±0.50**	**19.17±0.86**	**18.70±0.52**	**19.98±0.91**	**19.33±0.51**	**20.56±0.92**	**20.01±0.54**	**21.14±0.91**	**20.85±0.58**	**21.70±0.91**

Superscripts are read columns wise for comparison of mean. Different superscripts differed significantly ($p< 0.05$)

Table No. 10 Effect of duration of post-thaw incubation at 37^0 C on Acrosomal Damage (%) in Spring and Summer

Bulls	Acrosomal Damage%									
	0 min		15 min		30 min		45 min		60 min	
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer
Bull No 429	18.10^{a}±0.87	20.40^{a}±0.80	18.70^{a}±0.94	21.30^{a}±1.11	19.20^{a}±0.92	22.50^{ab}±1.30	20.00^{a}±0.88	23.30^{ab}±1.43	20.70^{a}±0.90	24.20^{ab}±1.66
Bull No 525	19.90^{a}±1.36	25.70^{b}±1.02	21.10^{a}±1.41	27.10^{b}±2.06	21.70^{a}±1.39	28.30^{c}±2.08	22.10^{a}±1.41	29.50^{c}±2.16	22.70^{a}±1.45	30.90^{c}±2.46
Bull No 560	20.00^{a}±1.02	21.40^{ab}±0.60	21.00^{a}±1.05	22.20^{ab}±0.64	21.50^{a}±1.04	23.10^{ab}±0.65	22.20^{a}±1.08	23.80^{ab}±0.55	23.00^{a}±1.10	24.80^{ab}±0.55
Bull No 555	20.60^{a}±1.65	22.50^{ab}±2.04	21.30^{a}±1.64	23.20^{ab}±2.11	22.00^{a}±1.69	24.30^{bc}±2.17	22.60^{a}±1.70	25.2^{abc}±2.20	23.10^{a}±1.68	27.70^{bc}±2.42
Bull No 570	19.20^{a}±1.51	25.71^{b}±1.63	20.30^{a}±1.62	26.85^{b}±1.64	20.70^{a}±1.61	27.28^{bc}±1.60	21.50^{a}±1.76	28.00^{bc}±1.74	22.20^{a}±1.71	29.42^{bc}±2.15
Bull No 559	16.00^{a}±1.93	18.50^{a}±1.24	16.70^{a}±2.04	19.40^{a}±1.34	17.40^{a}±2.07	20.00^{a}±1.25	17.90^{a}±2.06	20.70^{a}±1.25	18.30^{a}±2.24	21.80^{a}±1.22
Overall mean	**18.96±0.59**	**22.19±0.67***	**19.88±0.62**	**23.15±0.71***	**20.41±1.53**	**24.08±0.73***	**21.05±0.63**	**24.92±0.75**	**21.66±0.65**	**26.31±0.84***

Superscripts are read columns wise for comparison of mean. Different superscripts differed significantly ($p< 0.05$)
* Significant ($p<0.05$) between season

Table No. 11 Effect of duration of post-thaw incubation at 37^0 C on Hypo-osmotic sperm (%) in Spring and Summer

Bulls	Hypo-osmotic positive sperm percent							
	15 min		30 min		45 min		60 min	
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer
Bull No 429	$5.60^{a}\pm0.71$	$7.60^{b}\pm1.02$	$12.70^{a}\pm1.12$	$14.8^{b}\pm1.27$	$25.40^{a}\pm1.82$	$23.40^{ab}\pm1.77$	$42.10^{a}\pm2.12$	$48.30^{b}\pm2.99$
Bull No 525	$6.20^{a}\pm0.62$	$5.20^{ab}\pm1.07$	$13.10^{a}\pm0.70$	$10.3^{a}\pm1.22$	$25.20^{a}\pm1.59$	$19.20^{a}\pm1.54$	$41.40^{a}\pm2.87$	$27.60^{a}\pm1.96$
Bull No 560	$6.40^{a}\pm0.52$	$4.80^{a}\pm0.59$	$13.20^{a}\pm0.72$	$9.80^{a}\pm1.19$	$24.40^{a}\pm1.34$	$18.30^{a}\pm1.64$	$40.20^{a}\pm2.09$	$36.20^{b}\pm2.50$
Bull No 555	$5.60^{a}\pm0.65$	$6.80^{ab}\pm0.62$	$12.20^{a}\pm1.05$	$14.90^{b}\pm1.25$	$22.00^{a}\pm1.75$	$26.30^{b}\pm2.42$	$36.40^{a}\pm3.25$	$40.50^{b}\pm3.56$
Bull No 570	$6.10^{a}\pm0.31$	$5.42^{ab}\pm0.72$	$14.10^{a}\pm0.52$	$12.28^{ab}\pm1.71$	$25.60^{a}\pm1.44$	$22.40^{ab}\pm2.69$	$39.60^{a}\pm2.34$	$39.85^{b}\pm3.25$
Bull No 559	$5.70^{a}\pm0.49$	$5.40^{ab}\pm0.33$	$14.30^{b}\pm0.93$	$11.80^{ab}\pm0.38$	$24.80^{a}\pm1.33$	$23.6^{ab}\pm0.83$	$42.20^{b}\pm2.82$	$44.10^{b}\pm2.10$
Overall Mean	**5.93±0.22**	**5.89±0.33**	**13.26±0.35**	**12.31±0.53**	**24.56±0.62**	**22.19±0.81**	**40.31±1.05**	**38.61±1.32**

Superscripts are read columns wise for comparison of mean. Different superscripts differed significantly ($p< 0.05$)

Table No.12. Effect of duration of post-thaw incubation at 37^0 C on Cervical Mucus Penetration distance (mm) in Spring and Summer

Bulls	Cervical mucus penetration distance (mm)							
	15 min		30 min		45 min		60 min	
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer
Bull No 429	$5.30^{b}\pm0.39$	$4.60^{a}\pm0.52$	$10.50^{b}\pm0.68$	$9.50^{b}\pm0.76$	$16.40^{b}\pm0.95$	$14.40^{b}\pm1.11$	$22.80^{a}\pm2.12$	$20.60^{b}\pm1.55$
Bull No 525	$4.80^{b}\pm0.39$	$3.40^{a}\pm0.22$	$9.70^{ab}\pm0.89$	$6.80^{a}\pm0.51$	$15.00^{ab}\pm1.21$	$10.10^{a}\pm0.69$	$20.10^{a}\pm1.59$	$13.80^{a}\pm0.90$
Bull No 560	$4.90^{b}\pm0.37$	$3.60^{a}\pm0.42$	$9.60^{ab}\pm0.67$	$6.90^{a}\pm0.72$	$14.70^{ab}\pm0.85$	$10.10^{a}\pm1.026$	$21.30^{a}\pm1.44$	$14.10^{a}\pm1.38$
Bull No 555	$5.30^{b}\pm0.61$	$4.30^{a}\pm0.59$	$10.40^{b}\pm0.95$	$8.90^{ab}\pm1.13$	$15.40^{ab}\pm1.23$	$13.40^{ab}\pm1.49$	$20.60^{a}\pm1.77$	$18.10^{ab}\pm1.96$
Bull No 570	$4.00^{ab}\pm0.25$	$3.10^{a}\pm0.34$	$8.20^{ab}\pm0.35$	$6.71^{a}\pm0.47$	$13.00^{a}\pm0.55$	$10.42^{a}\pm0.51$	$19.20^{a}\pm0.99$	$14.28^{a}\pm0.83$
Bull No 559	$3.20^{a}\pm0.35$	$3.60^{a}\pm0.45$	$7.60^{a}\pm0.81$	$8.30^{ab}\pm0.71$	$12.50^{a}\pm1.07$	$13.10^{ab}\pm0.88$	$18.90^{a}\pm1.99$	$18.10^{ab}\pm1.08$
Overall Mean	**4.58±0.19**	**3.80±0.18**	**9.33±0.32**	**7.91±0.33**	**14.50±0.43**	**12.00±0.47**	**20.40±0.63**	**16.61±0.64**

Superscripts are read columns wise for comparison of mean. Different superscripts differed significantly ($p< 0.05$)

Table No. 13. Effect of level of LN_2 and duration of storage on post-thaw motility (%) and Hypo-osmotic swelling percent of Murrah buffalo bulls in spring and summer

Duration		Motility %				Hypo-osmotic swelling percent			
	Level	Full	3/4	1/2	¼	Full	3/4	1/2	¼
24 hrs	Spring	64.83±0.68	59.83±0.68*	50.41±0.79*	47.78±5.85*	43.87±1.04	41.80±1.08	38.60±0.96*	31.68±0.67*
	Summer	58.64±1.34	54.23±0.76**	48.25±1.21**	41.25±0.78**	41.85±1.32	37.25±0.87	31.21±0.51**	26.24±1.04**
48 hrs	Spring	64.83±0.68	55.50±0.74*	42.25±6.06*	35.75±0.74*	43.87±1.04	38.90±1.10	31.90±0.84*	24.55±0.61*
	Summer	58.64±1.34	49.38±0.53**	37.05±0.88**	29.27±1.23**	41.85±1.32	34.03±0.86	26.24±0.62**	19.85±1.20**
72 hrs	Spring	64.83±0.68	50.58±0.86*	33.66±0.86*	26.16±0.77*	43.87±1.04	36.11±1.08	25.56±0.75*	17.90±0.63*
	Summer	58.64±1.34	44.05±0.94**	28.24±0.12**	21.15±0.74**	41.85±1.32	30.17±0.82	20.12±1.07**	14.50±0.17**
96 hrs	Spring	64.83±0.68	44.58±1.03*	25.00±0.90*	16.66±0.74*	43.87±1.04	32.65±1.08*	19.16±0.71*	10.55±0.58*
	Summer	58.64±1.34	39.58±0.13**	21.14±0.23**	10.02±0.25**	41.85±1.32	27.12±0.27**	12.84±1.04**	7.02±1.08**
120 hrs	Spring	64.83±0.68	39.95±1.05*	19.03±0.83*	8.43±0.53*	43.87±1.04	28.76±1.06*	12.85±0.70*	4.26±0.41*
	Summer	58.64±1.34	34.07±1.14**	14.21±1.20**	4.24±1.21**	41.85±1.32	24.20±0.45**	9.12±0.24**	2.01±1.23**

* Significant ($p<0.05$) spring season
** Significant ($p<0.05$) summer season

Table No. 14. Effect of level of LN_2 and duration of storage on abnormal sperm (%) and Acrosomal damage of Murrah buffalo bulls in spring and summer

Duration		Abnormal sperm (%)				Acrosomal damage (%)			
	Level	Full	3/4	1/2	¼	Full	3/4	1/2	¼
24 hrs	Spring	18.11±0.50	18.41±0.49	19.33±2.05	18.85±0.48	18.96±0.59	19.80±4.67	21.03±0.61	23.30±0.61*
	Summer	19.17±0.86	19.21±0.12	20.22±0.47	21.27±1.14	22.19±0.67	24.27±1.29	27.25±1.34	32.02±1.09**
48 hrs	Spring	18.11±0.50	18.73±0.50	20.05±0.48	19.03±0.49	18.96±0.59	19.95±0.60	24.03±0.61	27.20±0.61*
	Summer	19.17±0.86	20.12±0.54	21.24±1.04	22.24±1.21	22.19±0.67	25.26±1.13	30.12±1.07	33.12±1.23**
72 hrs	Spring	18.11±0.50	19.26±0.50	19.37±0.27	19.51±0.49	18.96±0.59	20.63±0.61	27.10±0.66	31.23±0.64*
	Summer	19.17±0.86	20.08±1.24	21.57±1.09	22.86±0.60	22.19±0.67	28.25±2.05	32.25±1.28	35.26±1.51**
96 hrs	Spring	18.11±0.50	19.71±0.51	20.38±0.50	20.01±0.49	18.96±0.59	21.40±0.61	30.10±0.74*	35.16±0.65*
	Summer	19.17±0.86	20.15±0.89	21.34±0.63	22.97±1.24	22.19±0.67	28.76±1.08**	33.25±1.21**	38.05±1.05**
120 hrs	Spring	18.11±0.50	20.11±0.51	20.78±0.51	20.56±0.50	18.96±0.59	22.28±0.61	33.13±0.82*	39.05±0.70*
	Summer	19.17±0.86	20.45±1.20	22.23±0.95	22.18±0.59	22.19±0.67	31.08±1.12**	37.05±1.24**	45.07±1.27**

* Significant ($p<0.05$) spring season
** Significant ($p<0.05$) summer season

Table No. 15. Effect of level of LN_2 and duration of storage on cervical mucus penetration distance (mm) of Murrah Bulls in Spring and Summer

Duration	Level	Cervical mucus penetration distance (millimeter)			
		Full	3/4	1/2	¼
24 hrs	Spring	21.66±0.64	20.41±0.62	18.96±0.61	17.81±0.55
	Summer	17.59±0.64	17.37±1.21	16.04±1.08	14.28±1.21
48 hrs	Spring	21.66±0.64	18.86±0.60	15.53±0.51	13.31±0.45
	Summer	17.59±0.64	17.01±1.08	13.25±0.52	12.07±1.24
72 hrs	Spring	21.66±0.64	16.66±0.59	12.15±0.44	9.08±0.36**
	Summer	17.59±0.64	15.21±0.78	10.24±1.04	7.02±1.31*
96 hrs	Spring	21.66±0.64	14.48±0.59*	9.11±0.38*	5.25±0.30**
	Summer	17.59±0.64	13.78±0.45**	7.12±0.15**	3.02±0.24*
120 hrs	Spring	21.66±0.64	10.40±0.50*	6.20±0.33*	2.08±0.16**
	Summer	17.59±0.64	8.59±0.23**	5.01±0.44**	1.54±0.78*

* Significant ($p<0.05$) spring season
** Significant ($p<0.05$) summer season

Table No 6. Seminal Characteristics of frozen semen of Murrah bulls during spring and summer season

Bulls	Season	Prefreeze motility	P.T. motility	Live sperm (%)	HOST (%)	CMPT (mm)	Abnormal sperm (%)	Acrosomal abnormality(%)
Bull No 429	Spring	$82.50^{b}\pm1.11$	$68.50^{c}\pm1.50$	$64.40^{ab}\pm2.22$	$45.80^{a}\pm2.07$	$23.80^{a}\pm1.28$	$16.70^{ab}\pm1.13$	$18.10^{a}\pm0.87$
	Summer	$77.00^{b}\pm1.33$	$61.00^{c}\pm1.24$	$66.60^{b}\pm2.04$	$47.45^{b}\pm3.06$	$21.60^{c}\pm1.48$	$15.30^{a}\pm1.47$	$20.40^{a}\pm0.80$
Bull No 525	Spring	$77.00^{a}\pm1.52$	$63.50^{ab}\pm1.06$	$61.50^{a}\pm2.91$	$45.40^{a}\pm2.66$	$21.40^{a}\pm1.57$	$17.70^{ab}\pm1.27$	$19.90^{a}\pm1.36$
	Summer	$72.50^{b}\pm4.29$	$57.20^{ab}\pm4.18$	$52.70^{a}\pm1.89$	$31.50^{a}\pm2.19$	$14.70^{a}\pm0.95$	$26.80^{b}\pm2.23$	$25.70^{b}\pm1.02$
Bull No 560	Spring	$76.50^{a}\pm1.30$	$63.00^{ab}\pm1.10$	$63.60^{ab}\pm1.80$	$44.20^{a}\pm1.85$	$22.70^{a}\pm1.39$	$19.30^{ab}\pm1.13$	$20.00^{a}\pm1.02$
	Summer	$71.50^{b}\pm3.07$	$59.10^{b}\pm2.50$	$54.10^{a}\pm1.30$	$40.45^{b}\pm2.75$	$15.10^{ab}\pm1.28$	$17.60^{a}\pm1.27$	$21.40^{ab}\pm0.60$
Bull No 555	Spring	$78.50^{ab}\pm1.50$	$69.00^{c}\pm1.94$	$63.35^{ab}\pm2.11$	$40.10^{a}\pm3.49$	$22.00^{a}\pm1.84$	$20.10^{b}\pm1.32$	$20.60^{a}\pm1.65$
	Summer	$70.00^{ab}\pm4.15$	$60.50^{b}\pm4.04$	$52.00^{a}\pm3.50$	$44.20^{b}\pm3.61$	$19.30^{bc}\pm1.97$	$19.20^{a}\pm2.32$	$22.50^{ab}\pm2.04$
Bull No 570	Spring	$75.00^{a}\pm1.66$	$59.50^{a}\pm1.16$	$61.90^{a}\pm2.45$	$42.25^{a}\pm2.29$	$20.00^{a}\pm1.15$	$19.30^{ab}\pm1.14$	$19.20^{a}\pm1.51$
	Summer	$60.71^{a}\pm4.29$	$49.28^{a}\pm4.29$	$51.57^{a}\pm2.85$	$40.57^{b}\pm3.51$	$15.14^{ab}\pm0.83$	$20.20^{a}\pm1.42$	$25.71^{b}\pm1.63$
Bull No 559	Spring	$77.50^{a}\pm1.34$	$65.50^{bc}\pm1.16$	$70.90^{b}\pm3.29$	$45.45^{a}\pm2.83$	$20.10^{a}\pm2.07$	$15.60^{a}\pm1.09$	$16.00^{a}\pm1.93$
	Summer	$76.00^{b}\pm2.56$	$62.00^{c}\pm2.49$	$60.20^{bc}\pm1.62$	$46.60^{b}\pm1.69$	$19.00^{abc}\pm1.22$	$16.40^{a}\pm1.38$	$18.50^{a}\pm1.24$
Overall Mean Spring		77.83±0.63	64.83±0.68	64.27±1.06	43.87±1.04	21.66±0.64	18.11±0.50	18.96±0.59
Overall Mean summer		71.84±1.51*	58.64±1.34*	56.43±1.15*	41.85±1.32	17.59±0.64*	19.17±0.86	22.19±0.67*

Superscripts are read columns wise for comparison of mean. Different superscripts differed significantly ($p< 0.05$)

* Significant ($p<0.05$) between season