STUDY ON FLUNIXIN MEGLUMINE TO IMPROVE CONCEPTION FOLLOWING EMBRYO TRANSFER IN CATTLE

Thesis

Submitted to

G.B. PANT UNIVERSITY OF AGRICULTURE & TECHNOLOGY, PANTNAGAR-263 145 (U.S. NAGAR), UTTARAKHAND, INDIA

By

Ankit Malik
(B. V. Sc & A.H.)

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Master of Veterinary Sciences
(Veterinary Gynaecology & Obstetrics)

AUGUST, 2017
ACKNOWLEDGEMENT

I bow my head in the lotus feet of almighty in providing me immense blessing and potency for completion of this mission.

First of all, I bow to the lotus feet of Parents and thanks to my sister who has been giving me courage, patience motivation and love throughout the course of study and life.

I express deepest gratitude and appreciation to the Chairman of my Advisory Committee, my supervisor, Dr. Shiv Prasad, Professor and Head, Department of Veterinary Gynaecology and Obstetrics, who has supported me throughout my thesis with his patience and knowledge whilst allowing me the room to work in my own way. I attribute the level of my Masters degree to his guidance, support, encouragement, effort and fatherly affection and without him this thesis, would not have been completed or written. Under his guidance I successfully overcame many difficulties and learned a lot. I can’t forget all the hard times that many times made me feel down, if it was not him supporting me through all the ups and downs of my life, my thesis would never had taken any shape nor would have I been able to be what I am. Despite of his busy schedule, he used to be available to review my thesis progress, give his valuable suggestions and made corrections. His unflinching courage and conviction will always inspire me. I am indeed privileged to have been guided and groomed under a dexterous gynaecologist of unchallenged repute. It was his unbeatable confidence in me that kept me motivated to overcome all challenges. Words are less to express my reverence to my respected guide, all I can say at this moment is that am grateful to the almighty for blessing me under his guidance. This thesis is a small tribute to an exceptional man from a student still anxious to learn from him.

I take it as great privilege to express my deep sense of gratitude for the honourable members of my advisory committee Dr. H.P. Gupta, Professor, Dr. Mridula Sharma, Assistant Professor, Department of Veterinary Gynaecology and Obstetrics, Dr. N.S. Jadon, Professor and Head, Department of Veterinary Surgery & Radiology, Dr. S.K. Rastogi, Professor, Department of Veterinary Physiology and Biochemistry for their invaluable inspiration, consistent help, guidance with constructive suggestions, deliberative discussion and active persuasion throughout the course of my study.

I express my thanks to Dr. V.S. Rajora, Professor, Department of Veterinary Medicine, Dr. A.K. Das, Professor, Department of Veterinary Surgery & Radiology, Dr. Nidhi Arora, Associate Professor, Department of Veterinary Medicine, Dr. Meena Mrigesh, Department of Veterinary Anatomy, Dr. R. Huozha, Dr. Sudhir Kumar, Assistant Professor, Department of Veterinary Physiology and Biochemistry for their help and support and Goodwill.

I express my special thanks and gratitude to Dr. Sunil Kumar, Assistant Professor, Department of Veterinary Gynaecology and Obstetrics, for his valuable support, consistent help and encouraging me during this investigation.

I extend my lot of thanks to my best buddy (motuu..) who always made me feel stronger by boosting up my moral and made me more determined to achieve my goals. I
veraciously realized the inadequacy of words for your continuous encouragement in bringing me to this stage of my life.

I must acknowledge Dean, Post Graduate Studies; Dean, College of Veterinary and Animal Sciences, Director Experiment Station, Joint Director Dairy Farm (I.D.F) for providing me necessary facilities and making the environment conducive to conduct this investigation.

I am highly grateful to Dr. Taru Sharma, Principal Scientist and Head cum Director, Center for Advanced Studies in Veterinary Physiology, Division of Physiology and Climatology, I.V.R.I., Izatnagar, Bareilly for providing RIA facilities in his laboratory and his valuable help, love and affection. I am also thankful to Mr. M.C. Pathak Senior Technical Officer, Nuclear Research Laboratory (NRL), I.V.R.I. for his valuable help.

I wish to pay my regards to seniors Drs. Bhoopendra, Raj, Arun, Rashmi, Ram Krishna, Nitin, and Maleeha for their constant support and encouragement.

A fervent thanks to my friends Drs. Raman, Chirag, Kunal, Suyash, Devendar, Yaquub, Kanika, Prathiba, Pushpsa, Rakesh, Mayank, Komal, Neha and Anand whose constant encouragement gave me impetus to fulfill this mammoth task.

I express my thanks to my juniors Drs. Dwipijyoti, Nitin, Nitish, Parul, Vidhi, Suchitra, Tshering, Surya, Pawan, Vijay, Dinesh, and Vikash for providing humorous company and cooperation.

I am also thankful to S/Sri, C.B.S. Pandey, M.K. Nishad, Rajdev, Sahdev, Manoj Sunita Devi and Shanit Devi for their help in laboratory and day to day work.

Author express science thanks to Dr. Ramvir Singh, Mr Purusottam Sharma, Kamlesh, Purshpendar and Naeem for their constant help during entire period of research work in dairy.

Where emotions are involved worlds cease to mean. I also admit myself incapable to pay regards in words to the affection, blessings, encouragement and dedication of my parents. I would have never come to this stage and engaged myself in professional pursuit and carries building without their efforts. I am bereft of words to convey my emotions and gratitude to my beloved sister for her encouragement blended with love and affections during my thesis work.

I am also thankful to many persons helping me directly or indirectly during my study. It is difficult to thank all of them individually by name. This short coming may please be pardoned.

Last but by no means the least, I wish to pay my respects to the experimental animals used during this study and hope that the results of this study may in some small way recompense the difficulties they may have undergone during this work.

Pantnagar
August, 2017

(Ankit Malik)
Author
CERTIFICATE

This is to certify that the thesis entitled “STUDY ON FLUNIXIN MEGLUMINE TO IMPROVE CONCEPTION FOLLOWING EMBRYO TRANSFER IN CATTLE” submitted in partial fulfilment of the requirements for the degree of Master of Veterinary Science with major in Veterinary Gynaecology and Obstetrics and minor in Veterinary Surgery and Radiology of the College of Post Graduate Studies, G.B. Pant University of Agriculture and Technology, Pantnagar, is a record of bona fide research carried out by Dr. Ankit Malik, Id. No. 39811 under my supervision and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged.

Pantnagar
August, 2017

(Shiv Prasad)
Chairman
Advisory Committee
CERTIFICATE

We, the undersigned, members of the Advisory Committee of Dr. Ankit Malik, Id. No. 39811 a candidate for the degree of Master of Veterinary Science with major in Veterinary Gynaecology and Obstetrics and minor in Veterinary Surgery and Radiology agree that the thesis entitled “STUDY ON FLUNIXIN MEGLUMINE TO IMPROVE CONCEPTION FOLLOWING EMBRYO TRANSFER IN CATTLE” may be submitted in partial fulfilment of the requirements for the degree.

(Shiv Prasad)  
Chairman  
Advisory Committee

(H. P. Gupta)  
Member

(N. S. Jadon)  
Member

(S. K. Rastogi)  
Member

(Mridula Sharma)  
Member
<table>
<thead>
<tr>
<th>S. N.</th>
<th>Chapters</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Introduction</em></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td><em>Review of Literature</em></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td><em>Materials and Methods</em></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td><em>Results and Discussion</em></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td><em>Summary and Conclusion</em></td>
<td></td>
</tr>
</tbody>
</table>

*Literature Cited*

*Annexure*

*Vita*

*Abstract*
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Flunixin meglumine treatment and blood sampling schedule in recipient cow</td>
<td></td>
</tr>
<tr>
<td>3.2</td>
<td>Blood sampling protocol for biochemical parameters in embryo recipient</td>
<td></td>
</tr>
<tr>
<td>3.3</td>
<td>Blood sampling protocol for PGFM in embryo recipient</td>
<td></td>
</tr>
<tr>
<td>3.4</td>
<td>Blood sampling protocol for progesterone in embryo recipient</td>
<td></td>
</tr>
<tr>
<td>4.1</td>
<td>Superovulatory response and embryo recovery in folltropin-V treated crossbred cows.</td>
<td></td>
</tr>
<tr>
<td>4.2</td>
<td>Superovulatory response in embryo donor cow following folltropin-V treatment (n=10).</td>
<td></td>
</tr>
<tr>
<td>4.3</td>
<td>Embryo/ova recovery rate in embryo donor cow following folltropin-V treatment (n=10).</td>
<td></td>
</tr>
<tr>
<td>4.4</td>
<td>Stages of embryo recovered from embryo donor cow following folltropin-V treatment (n=10).</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>Mean (±SE) serum Protein concentration (g/dl) of different groups on day of transfer (day 7), 16(^{th}), 17(^{th}), 18(^{th}) and 32(^{nd}) day post estrus in crossbred recipients.</td>
<td></td>
</tr>
<tr>
<td>4.6</td>
<td>Mean (±SE) serum glucose concentration (mg/dl) of different groups on day of transfer (day 7), 16(^{th}), 17(^{th}), 18(^{th}) and 32(^{nd}) day post estrus in crossbred recipients.</td>
<td></td>
</tr>
<tr>
<td>4.7</td>
<td>Mean (±SE) serum cholesterol concentration (mg/dl) of different groups on day of transfer (day 7), 16(^{th}), 17(^{th}), 18(^{th}) and 32(^{nd}) day post estrus in crossbred recipients.</td>
<td></td>
</tr>
<tr>
<td>4.8</td>
<td>Mean (±SE) serum creatinine concentration (mg/dl) of different groups on day of transfer (day 7), 16(^{th}), 17(^{th}), 18(^{th}) and 32(^{nd}) day post estrus in crossbred recipients.</td>
<td></td>
</tr>
<tr>
<td>4.9</td>
<td>Mean (±SE) serum urea concentration (mg/dl) of different groups on day of transfer (day 7), 16(^{th}), 17(^{th}), 18(^{th}) and 32(^{nd}) day post estrus in crossbred recipients.</td>
<td></td>
</tr>
</tbody>
</table>
4.10 Mean (±SE) serum PGFM concentration (pg/ml) of different groups on day of transfer (day 7), 16\textsuperscript{th}, 17\textsuperscript{th} and 18\textsuperscript{th} day of estrus in crossbred recipients

4.11 Mean (±SE) serum progesterone concentration (ng/ml) of different groups on day of transfer (day 7), 16\textsuperscript{th}, 17\textsuperscript{th}, 18\textsuperscript{th} and 32\textsuperscript{nd} day of estrus in crossbred recipients

4.12 Average serum PGFM concentration (pg/ml) in Control non-pregnant, Treated non-pregnant (T-II) and Treated pregnant/expected pregnancy (T-II) before and after transfer in crossbred recipient.

4.13 Average serum progesterone concentration (ng/ml) in Control non-pregnant, Treated non-pregnant (T-II) and Treated pregnant/expected pregnancy (T-II) before and after transfer in crossbred recipient.
<table>
<thead>
<tr>
<th>Fig. No.</th>
<th>Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Synthesis of prostaglandins</td>
<td></td>
</tr>
<tr>
<td>2.2</td>
<td>Change in blood PGFM concentration after embryo transfer</td>
<td></td>
</tr>
<tr>
<td>3.1</td>
<td>Protocol for embryo donor cow</td>
<td></td>
</tr>
<tr>
<td>3.2</td>
<td>Protocol for embryo recipient cow</td>
<td></td>
</tr>
<tr>
<td>4.1</td>
<td>Superovulatory response in embryo donor cow following folltropin-V treatment (n=10).</td>
<td></td>
</tr>
<tr>
<td>4.2</td>
<td>Embryo/ova recovery rate in embryo donor cow following folltropin-V treatment (n=10).</td>
<td></td>
</tr>
<tr>
<td>4.3</td>
<td>Stages of embryo recovered from embryo donor cow following folltropin-V treatment (n=10).</td>
<td></td>
</tr>
<tr>
<td>4.4</td>
<td>Mean (±SE) serum protein concentration (g/dl) of different groups on day of embryo transfer (day 7) at different intervals in crossbred embryo recipient.</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>Mean (±SE) serum protein concentration (g/dl) of different groups on 16th, 17th, 18th and 32nd day of estrus in crossbred embryo recipient.</td>
<td></td>
</tr>
<tr>
<td>4.6</td>
<td>Mean (±SE) serum glucose concentration (mg/dl) of different groups on day of embryo transfer (day 7) at different intervals in crossbred embryo recipient.</td>
<td></td>
</tr>
<tr>
<td>4.7</td>
<td>Mean (±SE) serum glucose concentration (mg/dl) of different groups on 16th, 17th, 18th and 32nd day of estrus in crossbred embryo recipient.</td>
<td></td>
</tr>
<tr>
<td>4.8</td>
<td>Mean (±SE) serum cholesterol concentration (mg/dl) of different groups on day of embryo transfer (day 7) at different intervals in crossbred embryo recipient.</td>
<td></td>
</tr>
<tr>
<td>4.9</td>
<td>Mean (±SE) serum cholesterol concentration (mg/dl) of different groups on 16th, 17th, 18th and 32nd day of estrus in crossbred embryo recipient.</td>
<td></td>
</tr>
<tr>
<td>4.10</td>
<td>Mean (±SE) serum creatinine concentration (mg/dl) of different groups on day of embryo transfer (day 7) at different intervals in crossbred embryo recipient.</td>
<td></td>
</tr>
<tr>
<td>4.11</td>
<td>Mean (±SE) serum creatinine concentration (mg/dl) of different groups on 16th, 17th, 18th and 32nd day of estrus in crossbred embryo recipient.</td>
<td></td>
</tr>
<tr>
<td>4.12</td>
<td>Mean (±SE) serum urea concentration (mg/dl) of different groups on day of embryo transfer (day 7) at different intervals in crossbred embryo recipient.</td>
<td></td>
</tr>
<tr>
<td>4.13</td>
<td>Mean (±SE) serum urea concentration (mg/dl) of different groups on 16th, 17th, 18th and 32nd day of estrus in crossbred embryo recipient.</td>
<td></td>
</tr>
</tbody>
</table>
4.14 Mean (±SE) serum PGFM concentration (pg/ml) of different groups on day of transfer (day 7) at different intervals in crossbred embryo recipient.

4.15 Mean (±SE) serum PGFM concentration (pg/ml) of different groups on 16th, 17th and 18th day of estrus in crossbred embryo recipient.

4.16 Mean (±SE) serum progesterone concentration (ng/ml) of different groups on 16th, 17th, 18th and 32nd day of estrus in crossbred embryo recipient.

4.17 Average serum PGFM concentration (pg/ml) in Control non-pregnant, Treated non-pregnant (T-II) and Treated pregnant/expected pregnancy (T-II) before and after transfer in crossbred recipient.

4.18 Average serum progesterone concentration (ng/ml) in Control non-pregnant, Treated non-pregnant (T-II) and Treated pregnant/expected pregnancy (T-II) before and after transfer in crossbred recipient.
### LIST OF IMAGES

<table>
<thead>
<tr>
<th>Image No.</th>
<th>Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Embryo flushing from crossbred cow</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Embryo transfer in crossbred cow</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Examination of superovulatory response using ultrasound</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Evaluation of embryo using stereozoom microscope</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Estimation of PGFM in blood serum using ELISA</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>ELISA plate before adding stop solution</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>ELISA plate after adding stop solution</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>4 cell stage embryo 4x magnification</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Morula 4x magnification</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Blastocyst at 4x magnification</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Blastocyst at 10x magnification</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Blastocyst at 10x magnification</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Blastocyst at 20x magnification</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Blastocyst at 40 x magnification</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Hatching blastocyst at 20x magnification</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Hatched zona 4x magnification</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Degenerated embryo at 4x magnification</td>
<td></td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

< : Less than
>
% : Per cent
&
/ : Per
@ : At the rate of
~ : approximately
≤ : Equal or less than
≥ : Equal or more than
β : Beta
°C : Degree Centigrade
µg : Microgram
µl : Microliter
AI : Artificial Insemination
CIDR : Controlled Intravaginal Drug Release Device
CL : Corpus Luteum
E₂ : Estradiol
EB-17β : Estradiol-17β
eCG : Equine Chorionic Gonadotropin
ELISA : Enzyme Linked Immuno Sorbent Assay
ET : Embryo Transfer
et al. : et alia (and others)
etc. : et cetera
ETT : Embryo Transfer Technology
FSH : Follicle Stimulating Hormone
FSH-P : Follicle Stimulating Hormone – Porcine
gm : Gram
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>GnRH</td>
<td>Gonadotropin Releasing Hormone</td>
</tr>
<tr>
<td>hCG</td>
<td>human Chorionic Gonadotropin</td>
</tr>
<tr>
<td>hMG</td>
<td>human menopausal gonadotropin</td>
</tr>
<tr>
<td>hr</td>
<td>Hour(s)</td>
</tr>
<tr>
<td>i.e.</td>
<td>id est (that is)</td>
</tr>
<tr>
<td>I.U.</td>
<td>International Unit(s)</td>
</tr>
<tr>
<td>IFN-τ</td>
<td>Interferon-tau</td>
</tr>
<tr>
<td>IGF 1</td>
<td>Insulin Like Growth Factor</td>
</tr>
<tr>
<td>IM</td>
<td>Intramuscular/ Intramuscularly</td>
</tr>
<tr>
<td>LH</td>
<td>Luteinizing Hormone</td>
</tr>
<tr>
<td>Lit</td>
<td>Liter</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>ml</td>
<td>millilitre</td>
</tr>
<tr>
<td>mm</td>
<td>Millimeter</td>
</tr>
<tr>
<td>mmol</td>
<td>Millimol</td>
</tr>
<tr>
<td>MOET</td>
<td>Multiple Ovulation and Embryo Transfer</td>
</tr>
<tr>
<td>NEB</td>
<td>Negative energy balance</td>
</tr>
<tr>
<td>NIH-FSH-P1</td>
<td>National Institute of Health – Follicle Stimulating Hormone- Porcine</td>
</tr>
<tr>
<td>NSS</td>
<td>Normal Saline Solution</td>
</tr>
<tr>
<td>Nm</td>
<td>Nanogram</td>
</tr>
<tr>
<td>P</td>
<td>Level of significance</td>
</tr>
<tr>
<td>P₄</td>
<td>Progesterone</td>
</tr>
<tr>
<td>PGF/PGF₂α</td>
<td>Postaglandin F</td>
</tr>
<tr>
<td>PMSG</td>
<td>Pregnant Mare Serum Gonadotropin</td>
</tr>
<tr>
<td>PRID</td>
<td>Progesterone Releasing Intravaginal Device</td>
</tr>
<tr>
<td>pg</td>
<td>Picogram</td>
</tr>
<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolution per minute</td>
</tr>
<tr>
<td>S/C</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>SOV</td>
<td>Superovulation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>SE</td>
<td>Standard error</td>
</tr>
<tr>
<td>SOET</td>
<td>Superovulation and embryo transfer</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of Mean</td>
</tr>
<tr>
<td>Std</td>
<td>Standard</td>
</tr>
<tr>
<td>TE</td>
<td>Total Embryo</td>
</tr>
<tr>
<td>TO</td>
<td>Total ova</td>
</tr>
<tr>
<td>VE</td>
<td>Viable Embryos</td>
</tr>
<tr>
<td><em>viz.</em></td>
<td>namely</td>
</tr>
<tr>
<td>Vs.</td>
<td>Versus</td>
</tr>
</tbody>
</table>
Introduction
India ranks first in milk production i.e. 155.5 million tones, accounting for 18.5% of total world production. Growth rate of milk production is about 6.26% whereas world milk it is about 3.1% at global level. The per capita availability of milk in India has increased from 176 grams per day in 1990-1991 to 337 grams per day in 2015-2016 (Department of Animal Husbandry, Dairying & Fisheries, Ministry of Agriculture, GoI). It is more than the world average of 294 gram per day during 2013. India has the largest bovine population in the world which is 299.6 millions according to livestock census 2012. However, the average milk production of our bovine is far below of the international standard. The reason for this disparity is low productivity of the animal in the tropics, poor genetic make-up and shorter lactation period. As a result of population explosion in the past decades, the gap between demand and supply of food of animal origin has further increased resulting into malnutrition in human beings.

Early embryonic mortality has been considered one of the major problem in cattle which is affecting dairy industry. The fertilisation rate in ranges between 55 and 98 % depending on the condition of the cow (Santos et al., 2004). The fertilisation rate approaches 100 per cent in heifers and the percentage of viable embryos decrease to about 70 per cent by sixth days after insemination. About 40 % of embryonic losses are likely to occur by between 8th and 17th days of pregnancy (Thatcher et al., 1994).

Embryo transfer technology (ETT) in cattle is being carried throughout world for the production of breeding stock, higher milk production and a calf per year. However, in spite of long time execution of this technology, establishment of pregnancy in recipient cattle remained lower and highly variable. This variation may be a result of embryonic, maternal or environmental factors or a combination of any of these factors (Sreenan and Diskin, 1987). ETT can be chosen to improve our livestock at faster rate. This technique, involves genetic contribution of both the male and female utilized simultaneously which leads to faster genetic improvement of the herd. In India and in other Asian countries, there has been an increasing demand of milk which could be fulfilled through production of elite germ. The application of embryo transfer technology is highly desirable to produce AI bulls from the best proven cows and bulls.
available. The first successful embryo transfer in cattle was done in 1951 by Willet et al. since then ETT has been improved considerably. Now a day’s ETT is being used as a routine method to produce improved breeding stock in different countries, but poor superovulatory response, improper storage and higher embryo mortality leading to lower conception rate in recipient animals, are some of the major problems limiting large scale field application of this technology.

In cattle, three or more than three ovulations are considered as superovulation (Bettridge et al., 1980). It is considered to be one of the most important steps in embryo transfer programme as it utilises the germ pool of higher genetic potential female and ensures the supply of embryos of good quality. Induction of superovulation for the first time has been reported by Casida et al. (1943). Since then many reports have been published (Monniaux et al., 1983; Baruselli et al., 2006, 2011; Bo and Mapleton, 2014). Gonadotropins are administered at mid luteal phase of the estrous cycle followed by an injection of prostaglandins 48-72 hours later to induce corpus luteum (CL) regression resulting in estrus and ovulation. Superovulation is an efficient technique for obtaining progeny from genetically valuable females. The ovarian response of each female depends on the number of gonadotropin-sensitive follicles present at the time of initiation of superovulatory treatment. One of the main factors influencing response to superovulation is stage of the follicular wave when gonadotropin treatments are given. Absence of dominant follicle generally enhances the superovulation treatment.

Follicle stimulating hormone (FSH) is a glycoprotein molecule having two sub units α and β. The α sub-unit is identical within species but β sub units is not specific and is responsible for the biological activity and species specific immune reaction. Since FSH has lesser sialic acid contents than PMSG which results in its shorter biological half life of 110 minutes. Therefore, repeated administrations are required for maintaining adequate concentration during super-ovulatory regimens for required duration (Bettridge, 1977).

FSH stimulates the growth and maturation of preantral and small antral follicles by initiation of granulosa cell mitosis, secretion of follicular fluid, antrum formation and by inducing granulosa cell sensitivity to LH by increasing the number of LH receptors. FSH also prevents some of the normal follicles from becoming atretic. FSH
treatment is initiated during mid luteal phase, around the time of emergence of the second follicular wave (Ginther et al., 1989) because the population of medium sized antral follicles has been reported to be higher during 9-13 days of estrous cycle. So, to maximize superovulatory response using FSH for embryo production, the timing of the first FSH treatment should be concurrent with first three days of the follicular wave emergence (Mutembei et al., 2015; Muasa et al., 2015). Therefore, treatment is initiated during mid luteal phase of estrous cycle. Generally traditional FSH treatment is given as double injection per day with dose ranging from 30-50 mg either for three days, four days or five days (Mapleton and Bo, 2012) or in increasing dose, constant dose or decreasing dose schedule. In recent years, many more alternative approaches for follicle wave synchronization and superovulation with FSH have been applied i.e. ultrasound guided follicle ablation (Bergfelt et al., 1997; Baracaldo et al., 2000), GnRH or pLH (Colazo et al., 2009) and by reducing the dose of FSH treatments (Lovie et al., 1994, Gath et al., 2012).

Successful pregnancy establishment is dependent upon the presence of a viable embryo in the uterine horn ipsilateral to a functional corpus luteum prior to the time of maternal recognition of pregnancy. The majority of embryo transfer occurs 6-7 days after a recipient cow has been detected in estrus. Generally, rectal palpation of the ovaries for the detection of the presence and the quality of a corpus luteum is followed by manipulation of the cervix and uterus to facilitate penetration of an embryo-bearing catheter into the lumen of a uterine horn ipsilateral to the corpus luteum. Placing the catheter and depositing the embryo as far as possible up the uterine horn appears to maximize the pregnancy rate.

Manipulation of the reproductive tract can cause trauma to the uterine endometrium causing inflammatory processes; owing to release of chemical mediators such as cytokines and prostaglandins. A 2-min uterine manipulation increases plasma concentration of 13,14-dihydro-15 keto prostaglandin 2α (PGFM) in cows at 35 days postpartum (Wann and Randel, 1990). The intensity of uterine manipulation following embryo transfer (ET) was scored on a scale from 1 (i.e., the transfer requiring minimum manipulation of the genital tract) to 3 (i.e., the transfer requiring extreme manipulation of the genital tract). Concentrations of PGF2α also increase following minimum manipulation during ET (Scenna et al., 2005). Both in vivo and in vitro
studies provided good affirmation that PGF2α has direct negative effects on embryonic survival or development (Buford et al., 1996; Seals et al., 1998).

However, placing the catheter through the cervical lumen, into the uterus, and up the uterine horn is very challenging in some cattle. Pregnancy rates may decrease in some embryo recipient cattle because manipulation of the uterus elevates levels of PGF2α in blood even minor manipulation of the cervix may lead to increased PGF2α in the lumen of the uterus. Increased PGF2α in the uterine lumen may interfere with embryonic development and affect embryo quality directly (Scenna et al., 2004). Alternatively, increased PGF2α in blood may indirectly affect pregnancy establishment by compromising luteal function (Beal et al., 1998).

During early pregnancy, interactions between the embryo and the maternal environment occur. A trophoblastic protein, IFN-τ, is produced by the embryo to prevent luteolysis induced by a pulsatile release of PGF2α from the uterus (Bazer, 1992; Demmers et al., 2001) and supports the recognition of pregnancy by the dam. This process of maternal recognition of pregnancy, occurring between the day 14th and 17th, pave the way to protect the corpus luteum from lutelytic effect of PGF2α and thus corpus luteum (Bazer et al., 1997) secretes adequate amount of progesterone which is essential for the establishing of pregnancy (Okuda et al., 2002). It is achieved by suppressing the oxytocin dependent pulsatile release of PGF2α from the endometrium through inhibiting the oxytocin receptor expression (Farin et al., 1990; Bazer et al., 1997). Pregnancy is associated with an increase in circulating progesterone concentration on day 4 or 5 after AI. One major problem that may arise is asynchrony between mother and embryo (Green et al., 2005). Delayed increase or an insufficient secretion of progesterone during the early luteal phase may cause slower embryonic growth (Kerbler et al., 1997; Mann et al., 1999; Mann and Lamming, 2001). If the conceptus has not continued to develop with the appropriate rate, it will not be able to produce sufficient IFN-τ to inhibit PGF2α synthesis (Mann and Lamming, 2001). The subsequent luteolysis interrupts the desired secretion of progesterone and results in embryonic loss during early pregnancy (Thatcher et al., 2001). Low progesterone during day 5 to 7 after AI has been associated with decreased fertility in dairy cows (Stronge et al., 2005). Lactating cows with milk progesterone concentrations more than 1.0 ng/ml within day 5 after AI and ≥2.0 ng/ml thereafter (i.e., until day 35 to 45
after AI) had a greater pregnancy rate (87%) as compared to the pregnancy rate (33%) of cows with insufficient progesterone concentration or a delayed increase in the level (Hommeida et al., 2004).

The present experiment has been designed with following objectives for improving conception following embryo transfer in crossbred cows.

1. To study the effect of flunixin meglumine on PGF2α, progesterone level following embryo transfer.
2. To study the alteration in glucose and protein in embryo recipient animals.
3. To study the effect of flunixin meglumine treatment on conception rate.
Non steroidal anti inflammatory drugs (NSAID) block the synthesis of prostaglandins, including PGF2α, via inhibition of the 2 isoforms of cyclooxygenase; COX-1 and COX-2 (Anderson et al., 1990). These enzymes are essential catalysts at the beginning of prostaglandin synthesis. This inhibitory effect has been used to delay luteolysis for 1 day in dairy heifers after a 7-day treatment period with flunixin meglumine (Aiumlamai et al., 1990). A potential positive effect on pregnancy rates through the application of NSAID has been hypothesized, particularly at the critical time of pregnancy recognition, between day 14 and 17 after breeding (Guzeloglu et al., 2007; Erdem and Guzeloglu, 2010). This could provide more time for the embryo to secrete sufficient IFN-τ for maternal recognition of pregnancy. Current evidence regarding the use of NSAID to improve pregnancy rates in cattle is controversial.

NSAIDs are the widely used drugs for the treatment of pain since centuries. These drugs also exhibit antipyretic and analgesic properties. They act by inhibiting the enzyme cyclooxygenase (hence also known as Cox inhibitors). This is how they block the synthesis of prostaglandins. Prostaglandins are mediators which ensure the formation of inflammatory symptoms as fever, pain and swelling. Arachidonic acid is a precursor of prostaglandins which is found in all cells within cell membrane.

![Synthesis of prostaglandins](image)

**Fig. 2.1: Synthesis of prostaglandins**
The end products of fatty acid metabolism are prostaglandins. Arachidonic acid is synthesized from membrane phospholipids, due to the effect of Phospholipase A2. As soon as arachidonic acid is released, Prostaglandin G₂ and Prostaglandin H₂ are synthesized by the effect of cox enzyme. After it, under the regulation of PG synthase enzyme, different other categories of prostaglandins; PGD₂, PGE₂, PGF₂, PGI₂ and TxA₂ are synthesized (Saraf, 2008; Salman et al., 2004; Botting, 2006; Rao and Knaus, 2008).

Aspirin is well known NSAID for the past hundreds years. The identification of salicylic acid in 1860 was the most important step in the discovery of aspirin. Following this discovery, sodium salicylate in 1875 and phenyl salicylate in 1886 were first used but these drugs produces serious side effects in gastrointestinal tract. Acetyl salicylic acid or Aspirin was discovered by Felix Hoffman in 1897. It was marketed under the name of Aspirin by Bayer Company in 1899 (Myers, 2003). For the first time, it has been reported that prostaglandin inhibitors prevent product of Cox by John Vane in 1971 (Botting, 2006). Later more studies have shown that Cox enzymes have different isoforms and have different functions. Cox-1 is found in stomach, intestine, kidney and thrombocytes, and Cox-2 is secreted in platelets, macrophages, endothelial cells (Isakson, 2003). While classic NSAIDs inhibit both enzymes, Cox-2 inhibitors inhibit inducible Cox-2. Cox-2 inhibitors can produce anti-inflammatory effect without forming any side effects in gastrointestinal system and in other tissues (Salman et al., 2004). Several other studies have been carried out to understand the importance of Cox enzyme in implantation. It has been found that COX-2 is produced by uterus luminal epithelium and stroma which surround blastocyst during implantation in rats. This indicates that COX-2 has a fundamental role in implantation (Reese et al., 1999; Chakraborty et al., 1996).

In another study, it has been seen that female rats which are having COX-1 deficiency have normal fertility and fecundity. Absence of COX-1 enzyme deficiency, COX-2 supplies this shortfall (Reese et al., 1999). But the, female rats having COX-2 deficiency are infertile because deficiency of COX-2 enzyme causes ovulation, fertilization, implantation and desidualization defects (Lim et al., 1997). Several other studies have been carried out on animals, to know the effects of NSAIDs on pregnancy rates in livestock. In these studies, flunixin meglumine, meloxicam, and carprofen have
been used in order to increase pregnancy rate in cows. Administration of ibuprofen lysinate, an inhibitor of prostaglandin synthesis, to embryo recipient dairy heifers 1 hour before embryo transfer increased pregnancy rates by 26% (Elli et al., 2001).

In an experiment with 10 lactating dairy cattle the manipulation of the reproductive tract during embryo transfer was followed by release of PGF2α from the uterus. Plasma concentrations of PGF2α at 10–60 and 130–230 min after uterine manipulation were higher than before uterine manipulation (Fig. 2.2). However, no differences were observed between concentrations of PGF2α from 70 to 120 min after uterine manipulation for transfer and before uterine manipulation, suggesting a pulsatile release of PGF2α.

Fig. 2.2: Change in blood PGFM concentration after embryo transfer

Concentrations of prostaglandin PGF2α before and after nonsurgical embryo transfer on day 7 after estrus. Values of PGF2α represent mean concentrations of PGF2α in blood samples collected every 10 min from 60 min before, 10–60 min after, 70–120 min after, and 130–230 min after embryo transfer. The blood concentrations of PGF2α after uterine manipulation reached 241.2 pg/ml and 249.7 pg/ml from 10 to 60 min and 130 to 230 min, respectively, following embryo transfer (with a transfer score of 1; minimal manipulation) Different letters (a–c) above individual bars indicate significance from PGF2α values collected before embryo transfer (Scennaa et al., 2005).
Similar to above study other studies have been also reported in other species, that a release of PGF2α following uterine manipulation in mares (Kask and Odensvik, 1995), sows (Kunavongkrit et al., 1984), and cows (Wann and Randel, 1990).

Interestingly it is seen that production of PGF2α during luteolysis and after the uterine manipulation followed a similar pulsatile release of PGF2α (Flint and Sheldrick, 1982). The endometrium of bovine contains comparatively large amounts of arachidonic acid that can be readily converted to different products such as PGF2α (Salamonsen and Findlay, 1990). Synthesis of PGF2α begins with the release of arachidonic acid from phospholipids membrane by the action of cytosolic phospholipase A2 (Gijon and Leslie, 1999). Free arachidonic acid is then converted to prostaglandin G2 (PGG2) and prostaglandin H2 (PGH2) by the action of the cyclooxygenase enzymes, COX-1 and COX-2. Lastly, prostaglandin F synthase mediates the formation of PGF2α from PGH2. Flunixin meglumine is a non-specific inhibitor of prostaglandin synthesis that means it will inhibit both COX-1 and COX-2 enzymes (Cheng, 1998; Campbell and Blikslager, 2000). Manipulation of the reproductive tract causes trauma to the uterine endometrium, which results into an inflammatory process. During inflammation, many chemical mediators such as several cytokines and prostaglandins are released at the site of trauma. One of the most important cytokines, tumour necrosis factor α (TNF-α), is capable of releasing PGF2α by luteal cells (Nothnick and Pate, 1900) and epithelial cells in the uterine endometrium via activation of PLA2 and nitric oxide synthase (Skarzynski et al., 2000). Moreover, (Okuda et al., 2002) proposed that endometrial TNF-α may be a trigger for production of PGF2α from the uterine endometrium during luteolysis. Since treatment of luteal cells with PGF2α initiate further production of PGF2α by this cell type in sheep (Wade and Lewis, 1996; Rexroad Jr and Guthrie, 1979) and pigs (Guthrie et al., 1979), PGF2α release after embryo transfer in cows may be able to stimulate its own production from the reproductive tract.

Even though after the release of PGF2α during embryo transfer may not result in luteolysis, embryonic survival may be compromised by the presence of small concentrations of PGF2α in the uterine lumen, creating a “hostile environment” for embryonic development. The development of in vitro produced 16–32-cell embryos to blastocyst stage was reduced by addition of 1 ng/ml of PGF2α in the culture media (Scenna et al., 2004). Furthermore, the same authors demonstrated that addition of 0.1,
1, and 10 ng/ml of PGF$_{2\alpha}$ to the culture media of in vivo derived compact morula did not affect development to blastocyst, but reduced hatching rates. In another study, addition of PGF$_{2\alpha}$ to culture media directly decreased the ability of eight-cell rabbit embryos to form expanded and hatched blastocysts, but had no effect on development to early blastocyst (Maurer and Beier, 1976).

The women undergoing in vitro fertilization and receiving a daily oral dose of 100 mg of aspirin (an inhibitor of COX-1 and COX-2 enzymes (Wu, 2003)) had better ovarian responsiveness (greater number of follicles and oocytes retrieved), uterine and ovarian blood flow velocity, implantation, and pregnancy rates than women receiving placebo (Rubinstein et al., 1999). In a similar by, Waldenstrom et al. (2004) reported higher pregnancy rates in women undergoing IVF and receiving 75 mg of aspirin daily from the day of embryo transfer until pregnancy test when compared to women receiving no treatment.

The negative effects of endometrial PGF2 released at Embryo Transfer on embryonic development and pregnancy rate can be overcome by local intrauterine administration of dexamethasone and recombinant human LIF (rhLIF) during embryo transfer (Sangho, 2016).

The secretions of progesterone during the first two weeks after the transfer have a decisive effect on the survival of bovine embryos. Presumably, steady concentrations of progesterone appeared to be favourable to retention of the transferred embryo (Cuadra et al., 2017).

2.1 Flunixin meglumine

The Chemical name of Flunixin meglumine is 3-Pyridinecarboxylic acid, 2-[[2-methyl-3-(trifluoromethyl)phenyl]amino]-, compounded with 1-deoxy-1-(methylamino)-D-glucitol (1:1) and Molecular formula is C$_{14}$H$_{11}$F$_{3}$N$_{2}$O$_{2}$ ⋅ C$_{7}$H$_{17}$NO$_{5}$, the molecular weight of Flunixin meglumine is 491.46. Description of Flunixin Meglumine USP is White to off-white crystalline powder, the pKa is 5.82 and is Soluble in water, in alcohol, and in methanol; practically insoluble in ethyl acetate.

Flunixin meglumine is a derivative of nicotinic acid and is also a non-selective cox inhibitor. It is a Non-narcotic, Nonsteroidal, potent and fast acting analgesic agent with anti-pyretic and anti-inflammatory activity. The precise site and mode of action is
unknown. Flunixin act via analgesic and anti-inflammatory mechanisms. Analgesic action may involve blocking pain impulse generation via a peripheral action by inhibition of synthesis of prostaglandins and possibly inhibition of synthesis or actions of other substances, which sensitize pain receptors to mechanical or chemical stimulation. Flunixin may act peripherally in inflamed tissue, probably by inhibiting the enzyme cyclooxygenase to decrease the formation of precursors of prostaglandins and probably by other local mediators of inflammatory response.

Protein binding in bovine plasma has been determined to be >99% over a concentration range of 3 to 24 micrograms per milliliter (mcg/mL) (Odensvik and Johansson, 1995).

Concentrations of Flunixin in cattle following a single intravenous dose of 2.2 mg per kg of body weight (mg/kg), plasma concentration was initially 16.16±28 mcg/mL, declined to 1.22±0.16 mcg/mL by 2 hours, and reached 0.5±0.02 mcg/mL by 30 hours. Following a single oral dose of 2.2 mg/kg, a peak concentration (Cmax) of 0.9±0.05 mcg/mL occurred 3.5±1.0 hours (Tmax) after the dose; serum concentration declined to 0.06±0.01 mcg/mL by 30 hours (Odensvik, 1995).

Mainly, it is used in visceral pains. In addition to its analgesic effect, it has antiendotoxic and antipyretic effects. It can be given at the dose rate of 1.1 mg to 2.2 mg per kg body weight or 1ml to 2 ml of flunixin injection per 45 kg body weight, by slow I/V or I/M injection route. Injection can be given either once a day as a single dose or divided into two doses and administered at 12 hours intervals. The half-life of Flunixin meglumine is between 8 and 12 hours in cows, but it is longer in other animals (Divers, 2008; Boothe, 2001). Flunixin meglumine is used in cows in combination with other antibiotics to cure illnesses like; joint ill, blackleg, transit fever, mastitis, puerperal metritis, vaginal prolapse, downer cow and pneumonia. It is also used as pain therapy after small operations (Scott et al., 2011; Braun, 2007). The routes of administration of flunixin meglumine in cows are intramuscular, intravenous and oral. The oral, dose is 1 mg/kg body weight and intravenous dose is 1.1-2.2 mg/kg body weight. Mostly intramuscular route is used and the dose is 1.1 mg/kg body weight. This dose of flunixin meglumine is given once in a day or twice in divided dose. Flunixin meglumine can be given at 6-8 hour intervals at the dose rate of 0.25-0.50 mg/kg body weight I/M. Average therapy period is for three days and it can be given for 5 days.
maximum (Radostits et al., 2006; Woolums et al., 2009; Blikslager and Jones, 2009; Mackay, 2009; Jones, 2009).

2.2 Superovulation

Superovulation is the process of induction of multiple ovulations above the level which occur naturally, using exogenous hormones. In cattle, which normally ovulate only one ovum, three or more ovulation are considered as superovulation (Betteridge, 1980). Superovulation was first time reported in cattle by Casida et al. (1943). Since then various reports on this aspect have been published (Hammond, 1950; Monniaux et al., 1983; Armstrong, 1993; Kharche et al., 2001).

The objective of superovulation is to obtain higher number of embryos eliciting a faster regenerator potential (Bo et al., 1996). However, superovulation is a major limiting factor in contribution that embryo transfer can make to genetic improvement of cattle. There are a number of parameters which influence the superovulatory response, like type of gonadotropin used (Monniaux et al., 1984; Pawlyshyn et al., 1986), lot of gonadotropin (Hill et al., 1986), breed of animal (Donaldson and Ward, 1984; Bindon et al., 1986; Munro, 1986; Kumar, 2002), age of animal (Hasler, 1983; Koll, 1984), nutrition status (Yaakub et al., 1999; Nolan et al., 1998), dose of FSH (Walton and Stubbing, 1986; Kudu et al., 1989), administration route and regimen of FSH (Hockley et al., 1992; Singh et al., 1997), presence of dominant follicle (Guibault et al., 1991; Romero et al., 1991), stage of estrous cycle at which superovulation treatment is started (Lindsell et al., 1986a), estrus synchronization method (Tenhumberg et al., 1984), flushing method (Sartori et al., 2003) etc.

Kweon et al., (1987) reported that status of CL (quantity of progesterone secretion at initiation of superovulatory treatment) is an important indicative of success of superovulation, the accuracy of predicting the number of recoverable embryos based on concentration of plasma progesterone was 86%.

There are a number of ways to superovulate domestic animals. Each has its advantages and disadvantages. The commonly used gonadotropin are equine chorionic gonadotropin (eCG, previously called PMSG), follicle stimulating hormone (FSH) and human menopausal gonadotropin (hMG) (Elsden et al., 1978; McGowan et al., 1985;
Bellows et al., 1991; Agarwal et al., 1993). There exist dispute regarding the superiority of one over the other.

The superovulatory treatment involves administration of gonadotropin during the midluteal phase of the estrus cycle followed by injection of prostaglandin (PGF$_{2\alpha}$) 48-72 hr later to induce luteal regression resulting in estrus and ovulation (Elsden et al., 1978; Monniaux et al., 1983; Shanker et al., 1991; Prasad, 2000).

2.3 Estrus Synchronization

The regulation of estrous cycle in cattle involves a number of hormones like progesterone, prostaglandin (PGF$_{2\alpha}$), gonadotropin releasing hormone (GnRH), follicle stimulating hormone (FSH) luteinizing hormone (LH) and estradiol-17β (EB-17β). Depending on the protocol, these hormones can be used for estrus synchronization independently or in combination with one another.

A few of these protocols that have been studied include the Monday Morning System – one shot prostaglandin (Sprott, 1999), two shot prostaglandin (Sprott, 1999; Stevenson et al., 2000), the MGS/Prostaglandin system (Wood et al., 2001), the MGA/GnRH/prostaglandin (Geary and Whittie, 1999; Lucy et al., 2001, Pharmacia Animal Health, 220).

Prostaglandin F$_{2\alpha}$ has been the most commonly used treatment for synchronization of estrus in cattle (Odde, 1990).

Estrous cycle is influenced not only by the responsiveness of the CL on the PGF treatment (Momont and Seguin, 1984) but also to stage of development of the dominant follicle at the time of PGF treatment (Kastelic and Ginther, 1991).

Earlier studies indicate that the maturity of the CL at the time of PGF$_{2\alpha}$ treatment influenced the luteolytic response and that PGF$_{2\alpha}$ did not effectively induce luteolysis during the first 5 or 6 day following estrus (Momont and Seguin, 1984). In cattle in which luteolysis did not occur, the ensuing estrus was distributed over a 6-day period (MacMillan et al., 1994), making fixed time AI programmes infeasible. If PGF is given when the dominant follicle of a wave is in the late growing or early static phase, ovulation will occur within 3 to 4 days. On the other hand, PGF treatment given in the mid to late growing or early static phases, (i.e. when it is no longer viable) will result in ovulation of the dominant follicle from the next follicular wave 5 to 7 days
later (Kastelic and Ginther, 1991). The phase of estrous cycle at the beginning of the treatment tended to influence the estrus. The best results were observed when the treatment began in the luteal phase (Roche, 1974; Mialot et al., 2002).

This interval is a reflection of the time required for the dominant follicle of the new wave to grow and develop to preovulatory state and further emphasized that both luteal and follicular control is required to obtain high pregnancy rate in fixed time AI or embryo transfer programme that does not require estrus detection.

The variation in size, shape and consistency of CL is common; therefore, estimation of CL size and number by palpation per rectum is not entirely reliable (Dawson, 1975; Duarant et al., 1986; Kelton et al., 1988).

When a single injection of cloprostenol was administrated, the degree of estrus synchronization was low (Cooper, 1974; King, 1982; Watts et al., 1985). However, in the study conducted by Silvia et al., (1984), no difference in effect on percentage of animal exhibiting estrus was detected following the 1\textsuperscript{st} and 2\textsuperscript{nd} injection of PGF\textsubscript{2α}.

2.4 Biochemical Profiles

2.4.1 Protein

The main physio-chemical function of the protein is in the maintenance of normal blood volume and water content in tissue fluids and assisting in solubility and transportation of lipids, bile salts, fat soluble vitamins and hormones in blood through formation of biochemical complexes (Roubicek and Ray, 1972). Successful embryo development depends upon the nature of the uterine environment. The uterine lumen is dynamic and exhibits marked differences between the stages of the estrous cycle as a consequence of ovarian steroidal regulation of endometrial secretion. Intake of high protein diets by lactating cows has been shown to alter the pH and the concentrations of other ions in uterine secretions, but only during the luteal phase and not at estrus. Uterine pH was also affected in heifers fed excess rumen degradable protein and was associated with reduced fertility (Butler, 1998). However, (Moallem et al. 2011) it is concluded that supplementation of high dietary crude protein for non-lactating heifer’s results in penetration of urea into pre-ovulatory follicles, but this does not impair the features of these follicles. Hence measurement of total protein level in blood circulation is necessary during superovulatory treatment and embryo development.
The mean values of total serum protein in cows reported by various scientists were 6.78 ± 0.14 gm% (Memon and Mullick, 1961), 5.40-7.00 gm% (Samad et al., 1980). The concentration of total proteins at superovulatory estrus in goats (Ishwar and Pandey, 1994) and cow (Prasad, 1990) did not vary significantly in comparison to control. However, a total protein concentration in superovulated cows was significantly higher in good responder as compared to non-responders (Prasad, 2000; Agrawal and Maurya, 2002). The plasma level of insulin was positively correlated with total proteins in post-partum beef cows (Dampney et al., 2013). The mean (±SEM) concentrations of serum total protein (g/L) measured at day 7 and the day of embryo recovery (DER) in single ovulated non-lactating dairy cows and superovulated lactating dairy cows were 67.58±2.38, 68.3±2.32 and 69.42±1.19, 69.42±1.21 respectively (Rasolomboahanginjatovo et al., 2013) which do not differ significantly.

Animals with high serum protein produced a significantly below average number of embryos suitable for transfer and there was no significant associations between transferable embryos (TE) and concentrations of total proteins (Chorfi et al., 2007). However, similar number of follicles and C.L. per cows, and total number of ova or embryo recovered from adequate protein fed (12.3%) or high protein diet (27.4%) (Garcia et al., 1994). Balance the ration for crude protein according to level of milk production. For high producing and early lactation cows 35% of the crude protein should be undegradable protein (Yasothai, 2014). High energy diet leads to increase steroid hormones and improving reproductive indices in dairy heifers (Pirestani and Maybodi, 2015).

2.4.2 Glucose

Glucose is most important metabolic regulator of reproductive tract function and it is able to alter the functioning of cells at different levels of the hypothalamus–pituitary gonadal axis. Decrease in glucose level decreases LH secretion in several species (Wade et al., 1996). Hypothalamic neurons can sense decrease level of glucose (Ohkura et al., 2000). In the ovary, glucose is essential for the maintenance of the quality of oocytes (Rato et al., 2012; Sutton-McDowall et al., 2010). It is seen in mice that glucose within the oocyte regulates meiotic maturation (Downs, 1995). It is also an important factor for the development of the embryo. Hyperglycemia results into profound reproductive and developmental consequences. GLUT1, GLUT3 and the
insulin-sensitive glucose transporter GLUT4, are expressed in the ovary in many species and their expression is regulated by intraovarian factors during follicular development, maturation and ovulation (Purcell and Moley, 2009). The GLUT content can be up-regulated in rat ovary following Gonadotropin use (Zhang et al., 2012). GLUTs act as glucose sensors in cells. Glucokinase is a hexokinase that phosphorylates glucose (Matschinsky, 1990), it is a necessary step for glucose metabolism. Some workers reported that in females, glucose is, transferred into the oocyte by cumulus cells via the GLUT system and then by gap junctions. It has been suggested that this intercellular pathway may partly mediate the effects of high-glucose availability on oocyte quality (Wang et al., 2012). Glucose is thus very essential for reproductive tract functions as well as it have been a central nutrient in metabolism. Its status is sensed locally in the cells of the hypothalamo–pituitary–gonadal axis by a number of intracellular mechanisms which are probably inter-related with each other.

Variations of blood glucose might be due to imbalance ration feeding. Increase feed intake and liver function of lactating cows has been associated with a higher metabolic clearance rate of steroid and other metabolic elements such as glucose when compared to non lactating cows (Sangsritavong et al., 2002). Plasma glucose concentration at estrus was significantly (P<0.05) lower as compared to day of PGF2α treatment in goat (Selvaraju et al., 2003). Concentrations of circulating blood glucose (P=0.001) were lower in severe negative energy balance (SNEB) relative to mild negative energy balance (MNEB) dairy cows (Fenwick et al., 2008). These differences were reflected in the positively energy balance calculations of the cow. The glucose levels (mg/dL) of Sahiwal heifers, cows and Jersey × Sahiwal cows ranged from 61.90±1.34 to 97.32±0.63, 58.61±1.20 to 96.9±0.65 and 59.26±0.58 to 113.33±0.71, respectively during their adaptability (Sreedhar et al., 2013). The mean (±SEM) concentrations of serum glucose (mmol/L) measured at day 7 and the day of embryo recovery (DER) in single ovulated non-lactating dairy cows and superovulated lactating dairy cows were 3.77±0.29, 3.97±0.33 and 4.07±0.18, 3.81±0.16 respectively (Rasolomboahanginjatovo et al., 2013) which do not differ significantly. Milk production increases faster than energy intake in the first 4 to 6 wk after calving.

High yielding cows will experience negative energy balance where blood concentrations of NEFA increase, while concentrations of insulin-like growth factor-I
(IGF-I), glucose, and insulin are low in such animals. If this condition persists longer, these changes in blood metabolites and hormones may compromise ovarian function and fertility. In addition, energy balance and dry matter intake can decrease plasma concentrations of progesterone (Vasconcelos et al., 2003; Villa-Godoy et al., 1988) and possibly interfering with follicle development and pregnancy maintenance.

Glucose metabolism appears to be a pivotal metabolic biomarker especially at blastocyst stage (Gardner et al., 2010). The male embryos developed faster than female embryos only in the presence of exogenous glucose and explained this fact by differential gene expression in male and female bovine embryos (Bredbacka and Bredbacka, 1996). The requirement of glucose for maturing oocytes and growing embryos appeared to be species specific with buffalo embryos requiring glucose throughout the in vitro maturation and culture steps (Kumar et al., 2012). The supplementation of glucose in synthetic oviductal fluid medium during in vitro culture of buffalo embryos for 48 hpi could lead to a higher percentage of cleavage among the male embryos (Gopikrishnan, et al., 2015).

2.4.3 Cholesterol

Blood cholesterol plays a very important role in the physiology of animal reproduction. Blood cholesterol concentration and steroid synthesis have been reported to have positive relationship with energy status of animals resulting in better reproductive performance (Shahukar et al., 1985).

The average serum cholesterol level were 165.11±34.00 mg% in lactating cows (Memon and Mullick, 1961), 148.69± 55.05 mg% with a range of 20-320 mg% in Holstein cows of six years of age (Roussel et al., 1982). The mean (±SEM) concentrations of serum cholesterol (mmol/L) measured at day 7 and the day of embryo recovery (DER) in single ovulated non-lactating dairy cows and superovulated lactating dairy cows were 98.5±1.63, 99.92±1.61 and 99.65±0.86,101.88±0.81,respectively (Rasolomboahanginjatovo et al., 2013) which do not differ significantly.

The high levels of cholesterol were recorded at superovulatory estrus than spontaneous estrus (Prasad, 1990). The number of embryos in cows with a total cholesterol level of less than 130 mg% was significantly less than those cows with total cholesterol of 130 mg% and higher (Kweon et al., 1987). Further, cows with a lower
concentration of cholesterol produced a significantly lesser number of embryos (Nakanishi et al., 1991). However, there was no significant association between transferable embryos (TE) and concentrations of total cholesterol (Chorfi et al., 2007). It is suggested that cows, should have blood cholesterol concentration of 100-170 mg% (Liepe, 1992). The plasma cholesterol concentrations (mmol/l) in superovulated heifers and cows at the time of embryo recovery (Day 7) were 2.8±0.1 and 5.8±0.3 respectively and the numbers of viable embryos were negatively correlated with cholesterol (Velazquez et al., 2005). Cows which do not have a normal cholesterol level in relation to their milk yield were unhealthy and did not show good ovarian response to hormonal treatment as compared to cows which had normal cholesterol level (Kweon et al., 1987). Concentrations of cholesterol change inversely to that of progesterone since cholesterol is a precursor for the synthesis of progesterone (Rizzo et al., 2016).

2.4.4 Creatinine

Plasma/Serum creatinine is derived, almost in its entirety, from the catabolism of creatine in muscle tissue as energy in the form of phosphocreatine (Gonzalez and Silva, 2006). The presence of this metabolite in the circulation is a physiological factor, because that is one of the catabolite protein metabolisms. The creatinine excretion via the kidney is performed, and the plasma creatinine levels reflect the rate of renal filtration (Gregory et al., 2004).

In hot environments, thermal stress is known to cause peripheral vasodilatation, to allow loss of body heat through sweating, and it can therefore reduce the blood flow to the internal organs. In addition, dehydration can also result in reduced blood flow to the kidneys. As a result of heat stress, the kidneys are unable to perform their normal function. The rate of excretion of creatinine is influenced by the glomerular filtration rate and creatinine is eliminated more easily than urea (Guyton and Hall, 1996). It is found that lower heat tolerant capacity of the crossbred cows results in slower elimination of serum creatinine from the body (Sreedhar et al., 2013).

The serum creatinine levels (mg/dL) of Sahiwal heifers, cows and Jersey × Sahiwal cows ranged from 1.25±0.07 to 9.81±0.13, 1.29±0.09 to 9.90±0.17 and 1.43±0.10 to 16.18±0.15 respectively during their adaptability (Sreedhar et al., 2013). The levels of serum creatinine in Nelore cattle in relation to age factor showed no
differences (p>0.05) and were inversely related, with advancing age led to a tendency to decreased concentrations of this metabolite, with values of 1.85±0.41 and 1.71±0.42 mg /dL for ages 0-12 and 12-24 months, respectively (Saraiva et al., 2014).

2.4.5 Urea

The intake of high dietary protein can result in elevated blood concentrations of ammonia, urea, or both, depending upon the balance of protein fractions present in the rumen and the availability of fermentable carbohydrates. Increased plasma or milk urea nitrogen concentrations are highly correlated with decreased fertility in cows (Butler, 1998; Westwood et al., 1998; Wittwer et al., 1999; Sawa et al., 2011). Plasma urea is inversely related to uterine luminal pH and sequential measurements in lactating cows have demonstrated that uterine pH is dynamically attuned to changes in plasma urea with a time lag of several hours (Butler, 1998). As a result of feeding diets high in crude protein, increased plasma urea concentrations may interfere with the normal inductive actions of progesterone on the microenvironment of the uterus and, thereby, cause suboptimal conditions for support of embryo development (Butler, 2000). However, a nonlinear relationship found between milk urea concentration and the probability of conception. Therefore, further research would be required for the decision on the possible use of milk urea concentration to improve reproductive performance in cows (Rehak et al., 2009).

In vitro studies of bovine endometrial cell cultures have shown that urea alters both the pH gradient across the polarized cells and increases secretion of prostaglandin that may interfere with embryo development and viability (Butler, 1998). Embryo quality and development was reduced in lactating cows fed excess rumen degradable protein, but embryo transfer and superovulation experiments in beef heifers found no detrimental effect of high dietary crude protein urea on embryo viability, fertilization rate, or embryo quality (Gath et al., 2012). Since the energy balance status of the cattle in the various studies was different, the effects of high dietary protein in lactating cows may exacerbate metabolic or hormonal mediated processes that would result in impaired embryo development. For example, the long-term effect of NEBAL might impair the health of preovulatory oocytes and follicles and reduce progesterone concentrations after ovulation (Britt, 1992), in addition to which some aspect of protein metabolism would further compromise successful embryo development.
Additional research on the interactions of energy balance and dietary protein metabolism that may impact embryo developmental processes should further aid our understanding of poor fertility in high producing dairy cows.

Urea has held its synthesis in the liver from ammonia due to the catabolism of amino acids and the recycling of ammonia in the rumen. Urea levels are analyzed in relation to the level of dietary protein and kidney function, and may be increased in cases of power failure due to impairment of rumen microbial nitrogen compounds in use for protein synthesis, increasing the amount of ammonia absorbed from the rumen (Gonzalez and Silva, 2006)

The concentrations of urea in the serum and uterine lavage fluid were affected by the superovulatory (SOV) treatment in the cow. In both compartments, the mean concentrations of urea were significantly higher at DER than day 7 and the DER of the control group (Rasolomboahanginjatovo et al., 2013). Increased serum urea impaired fertility and embryo growth and survival in dairy cattle because it is associated with low uterine pH and a suboptimal environment for embryo growth and development (Rhoads et al., 2006). However, the level of urea in the present study was still in the normal range (Rhoads et al., 2006) and was not associated with the number of transferable embryos (TE).

Urea concentration was found higher in the severe negative energy balance (SNEB) cows (P=0.075) (Fenwick et al., 2008). The blood urea levels (mg/dL) of Sahiwal heifers, cows and Jersey × Sahiwal cows ranged from 11.72±0.94 to 47.21±0.64, 11.70±0.65 to 45.44±0.42 and 14.00±0.58 to 63.99±0.41 respectively during their adaptability (Sreedhar et al., 2013). The concentration of insulin increased (P<0.05), while that of urea decreased as lactation progressed in Sanga cows (Damptey et al., 2013) and IGF-I concentration was negatively correlated with urea (Damptey et al., 2013). The mean (±SEM) concentrations of serum urea (mmol/L) measured at day 7 and the day of embryo recovery (DER) in single ovulated non-lactating dairy cows and superovulated lactating dairy cows were 5.33±0.31, 3.31±0.36 and 5.34±0.15, 5.11±0.18 respectively (Rasolomboahanginjatovo et al., 2013) which do not differ significantly. The plasma urea concentrations (mmol/l) in superovulated heifers and cows at the time of embryo recovery (Day 7) were 4.4±0.1 and 5.1±0.2 respectively and the number of viable embryos was negatively correlated with Urea (Velazquez et al., 2005).
2.5 Hormonal profiles

2.5.1 Prostaglandins

2.5.1a Prostaglandin effect on embryo recipients

PGF plays a delicate role in embryo recipients. Maintaining a unique balance of PGF is very necessary to achieve optimum pregnancy rates. PGF stimulates endometrial vascularity, blastocyst hatching, and embryo implantation (Van der Weiden et al., 1993; Dinchuck et al., 1995; Charpigny et al., 1997; Lim et al., 1997; Reese et al., 1999) so it is important that its effects are not totally removed. On the other hand, too much PGF may cause premature luteolysis or can be toxic to embryo (Seals et al., 1998). The goal is to control the release of PGF so that its actions are beneficial rather than to compromise the pregnancy. Therefore, the dose of the PGF inhibitor is very crucial for optimizing higher pregnancy rates. Too high dose may cause the negative effect, while too little dose may have no effect on pregnancy rate (Elli et al., 2001).

2.5.1b Prostaglandin effect on embryos

Embryo viability has detrimentally affected on exposure to high levels of PGF, for example, postpartum cows have a higher concentration of uterine PGF levels than normally cycling cows, as a result of which the embryos from cattle with high PGF levels were of lesser quality. Due to these findings PGF is thought to decrease embryonic survival in postpartum cows (Schrick et al., 1993). Embryos exposed to PGF during early development become arrested at the morula stage of development and may be particularly sensitive to the embryotoxic effects of PGF (Scenna et al., 2004; Fazio and Schrick, 1997; Hernandez-Fonseca et al., 1997). Re-emphasized this phenomenon, results showed that cows treated with PGF produced 64% embryos were at morula stage while the control group produced none, suggesting that PGF inhibits development beyond the morula stage. Additionally, the qualities of recovered embryos from the PGF treated cows were much lower than the control group. The cows supported these findings and showed that even they supplemented with exogenous progesterone and subsequently exposed to PGF that embryo development was inhibited (Hockett et al., 2004). Other species of animals has resulted in similar findings. The rabbit (Maurer and Beier, 1976) and rat (Breuel et al., 1993a) both showed that PGF
had an inhibitory effect on the development of embryos, however caution was emphasized in drawing comparisons among the species due to differences in embryonic development. Exactly how PGF inhibits embryo development is not known. It is possible that PGF binds with the receptor that leads to an increase in intracellular calcium (Wiltbank et al., 1989). This increase in calcium may interfere with the compaction process and prevent blastocele formation (Schrick et al., 1999). It is also possible that PGF diffuses across cell membranes and alters intracellular concentrations of calcium, thus disrupting the normal compaction process. Either way, PGF seems to prevent the normal compaction process which leads to poorly developed embryos. High levels of plasma PGF2α have been shown to decrease pregnancy rates in cows.

2.5.2 Progesterone

Progesterone is an important endocrine hormone for establishing and maintaining pregnancy and ensuring proper embryonic development. Corpus luteum is the major source of progesterone in the cattle. Elevated concentrations of maternal serum progesterone early in pregnancy have been associated with higher pregnancy rates and embryonic growth rates in cattle (Lonergan et al., 2007; Spencer et al., 2007). Further, cows with serum progesterone concentrations less than 5ng/mL on day 14 were more likely to lose their pregnancies (Maurer and Chenault, 1983) between days 28 and 42 (Kenyon et al., 2013). The early rise in progesterone concentration was associated with pregnancy success after embryo transfer (Kenyon et al., 2013). There appears to be a relationship between early conceptus development and both the rate and timing of the progesterone increase after conception (Carter et al., 2008; Kenyon et al., 2013). The peak level of progesterone is during the mid luteal phase of cycle and lowest level on the day of standing estrus (Booth et al., 1975).

Within the first few weeks of gestation, bovine embryos must secrete appropriate amounts of IFN-τ to inhibit the luteolytic mechanism and maintain secretion of progesterone required for further development. Associations between low maternal progesterone concentrations and pregnancy failure have been reported (Lonergan et al., 2007; Kenyon et al., 2013), whereas early progesterone supplementation improved pregnancy rates and advanced embryonic development (Carter et al., 2008; Forde et al., 2009). Progesterone exerts its effect on the embryo indirectly through actions on the endometrium.
The concentrations of progesterone remains less than 1ng/ml around the time of estrus (Diaz et al., 1986; Saxena and Gupta, 1992) which gradually increases from day 3 to 4 following estrus to a mean value of 4.5ng/ml and peak mean value of 6.7ng/ml around day 9 to 16 of the cycle (Robertson, 1972; Rajamahendran et al., 1976). Although considerable individual variation has been recorded in its level (Oyedipe et al., 1986). Most of the workers reported that highest value varies between 2 to 10ng/ml and lowest between 0.1 to 0.8ng/ml (Wettman et al., 1972; Booth et al., 1975).

Following the prostaglandin injection for induction of estrus during superovulation, the progesterone level decreased sharply within 16 to 48 hr to less than 1ng/ml (Lindsell et al., 1986a; Kweon et al., 1987). However, in certain animals progesterone fluctuates above 1ng/ml throughout the reported time of estrus. The higher progesterone concentration might be due to incomplete regression of C.L. (Lindsell et al., 1986a) or lutenization of follicles (Booth et al., 1975) and premature ovulation (Jensen et al., 1982).

Progesterone concentration on day 3 to 4 post-estrus upto the day of embryo recovery was found to be significantly correlated with the number of C.L. (Goto et al., 1987; Dinar and Srinan, 1988; Alekseenko et al., 1989; Chauhan et al., 1995) total embryo or ova recovered (Kweon et al., 1987; Goto et al., 1987) and number of fertilized and transferable embryos (Lindsell et al., 1986a; Alekseenko et al., 1989). There was no significant relationship identified between progesterone concentrations at the time of embryo transfer and conceptus length at recovery on day 17 (Barnwell et al., 2015). The mean (±SEM) concentrations of serum progesterone (ng/mL) measured at day 7 and the day of embryo recovery (DER) in single ovulated non-lactating dairy cows and superovulated lactating dairy cows; 2.11±2.0, 2.65±1.98 and 2.08±1.0, 12.16±0.98 ng/ml respectively (Rasolomboahanginjatovo et al., 2013).

2.6 Use of NASAID for improving conception rate

2.6.1 NSAID used in embryo transfer

NSAIDs were applied at different times and different doses before embryo transfer in cows and heifers aimed for preparing a suitable environment inside the uterus. In most of the studies, flunixin meglumine or ibuprofen applications were used before the embryo transfer to increase the pregnancy rate has been reported (Elli et al.,
The ibuprofen application increases implantation rates during embryo transfer in cattle. In 100 heifers, 5 mg/kg dose of ibuprofen intramuscular 1 hour before embryo transfer was given. Pregnancy rate in the treatment group reached 82% compared to 56% in control group (Elli et al., 2001).

Various reports are available regarding impact of Meglumine on pregnancy rate in animals. In one study where Meglumine at the dose rate of 500 mg, 2-12 minute before embryo transfer was used alone or in combination with CIDR showed pregnancy rates as 65%, 60.7%, 74.7% and 69.8% in non treated, CIDR inserted group, meglumine treated group and combination of meglumine + CIDR group respectively (Purcell et al., 2005).

In another study Flunixin meglumine was used at the rate of 10 ml of total dose, when injected to beef cattle 2-5 minutes before the embryo transfer, the pregnancy rate was higher in flunixin meglumine treated group as compared to control (51.1% vs. 63.8%) (Schrick et al., 2001). Ten ml total dose of flunixin meglumine was injected in heifers just before embryo transfer. In contrast to this, some workers also showed Pregnancy examination at 90 days after the embryo transfer revealed, no nonsignificant difference between the treated and control group (n=165) (50% vs. 45%) (McNaughtan, 2004).

Another worker has reported that when 500 mg flunixin meglumine was given intramuscular five minutes before embryo transfer in a study done on 39 brown Swiss cattle, showed lower pregnancy rate (50%) as compared to control group (52.6%) (Bulbul et al., 2010).

2.6.2 NSAID used after insemination

The usage of flunixin meglumine, carprofen and meloxicam at different times post insemination, decreased PGF2α release, increased luteal progesterone level helped in preventing early embryonic deaths (Heuwieser et al., 2011; Amiridis et al., 2009; Guzeloglu et al., 2007; Merrill et al., 2003). The improved pregnancy rates have been accomplished using NSAIDs (Guzeloglu et al., 2007; Merrill et al., 2003; Merrill et al., 2004; Merrill et al., 2007), in contrast to this some workers did not find any
noticeable improvement (Lucacin et al., 2010; Rabaglino et al., 2010; Geary et al., 2010).

To prevent early embryonic deaths in cows that were exposed to transportation stress, flunixin meglumine was given. Animals (n=80) were divided into 3 groups; control, stress (S) and stress + flunixin meglumine (SFM). Estrus was synchronized with MGA + PGF2\(\alpha\), insemination was done by observing the heat. Animals were exposed to stress 14\(^{th}\) days post insemination. Flunixin meglumine @ dose of 1.1 mg/kg intramuscular was given to SFM group before transportation. Only transportation stress was given in S group. The pregnancy rate was found to be (Control 76%, Stress 69% and SFM 84%), it has been seen that there is a positive relation between pregnancy rates and flunixin meglumine application (Merrill et al., 2003).

Flunixin meglumine administered intramuscular at a rate of 1.1mg/kg body weight to see the effect on embryonic mortality of stressed and unstressed cows. Angus heifers (n=259) and cows (n=127) were divided into groups as; control, control + flunixin meglumine, stress and stress + flunixin meglumine for two different trial in heifers and cows. In the first experiment, 259 crossbred Angus heifers were used. They were synchronized with Controlled Internal Drug-Release (CIDR®) and PGF2\(\alpha\). In the second experiment, 127 Angus crossbred cows were taken and synchronized with MGA and PGF2\(\alpha\). Flunixin meglumine was given in designed groups. The pregnancy rate of animals exposed to transportation stress was 62%. 14 days post artificial insemination, unstressed animals had pregnancy rate 64%. While the pregnancy rate of flunixin meglumine treated animals had 69% stressed, and 59% in unstressed, respectively. In the first experiment animals which were given flunixin meglumine had higher pregnancy rate than others in which flunixin meglumine was not given. In the second experiment, it was also seen that animals treated with flunixin meglumine had higher pregnancy rates (80%) than non treated (66%) (Merrill et al., 2004).

Flunixin meglumine injection at a dose rate of 1.1 mg/kg given intramuscular once on day 14 post insemination to animals that were exposed to transportation stress, to examine the effect of this drug on early embryonic deaths, prostaglandin and cortisol production in blood circulation. Beef cows (n=483) were divided into 4 different groups as; first group as transport, second group as transport + flunixin meglumine, third group as no transport and the last group as no transport + flunixin meglumine. It was observed
that, transport + flunixin meglumine group had higher pregnancy rate than non-treated group (74% vs. 66%) without considering the transportation. Only flunixin meglumine administered cows’ pregnancy rates were found to be higher than non-treated cows (71% vs. 61%). Cortisol level in cows exposed to transportation stress was found increased but pregnancy rate did not differ. In flunixin meglumine treated cows prostaglandin concentration was found to be lower than non-treated group. They concludes that NSAID applications would increase the pregnancy rate (Merrill et al., 2007).

The application of flunixin meglumine both orally and parentally supports luteal function. Orally flunixin meglumine at a dose rate of 2.2 mg/kg administered 2, 3 or 4 divided doses to heifers. The treatment was started on 14-15 days of the estrus. As a result, it was found that length of estrous cycle was increased in groups, in which flunixin meglumine was given in 3-4 divided doses. Luteolysis had taken place when 2 or 3 doses of flunixin meglumine had been given. But in group where flunixin meglumine was given in 4 doses luteolysis have been postponed (Odensvik et al., 1998).

In one study repeat breeder heifers were synchronised by PGF2α, the synchronisation was done by applying two dose of PGF2α 11 days interval, 48 hours after second PGF2α injection, GnRH (buserelin acetate) was administered and after 12–14 hours fixed time artificial insemination was done. Then the heifers were divided into two groups randomly and one group was treated with flunixin meglumine at the dose rate of 1.1 mg/kg body weight intramuscularly on the 15th day and 16th day and in second group no treatment was given and was treated as control. Pregnancy diagnosis was done on the 29th day, the pregnancy rate was 50% in treated group, and 20% in the control group (Dogruer et al., 2007).

In another similar study GnRH was given on the 48th hour after synchronisation with PGF2α in Holstein heifers (n=52) and artificial insemination was done after 12-14 hours. Flunixin meglumine was administered at the dose rate of 1.1 mg/kg body weight after artificial insemination on 15th day evening and 16th day morning via intramuscular route. Pregnancy diagnosis was done on the 29th day and, there were 20 pregnant animals in the treatment group and 13 pregnant animals in the control group (Guzeloglu et al., 2007).

In one of the studies, flunixin meglumine was administered at the dose rate of 1.1 mg/kg body weight to animals in between the estrus cycle on 11th and 16th day, and
saline solution was given in the control group. The estrus cycle was synchronized by the applications of estradiol benzoate + CIDR + PGF2α and then, fixed time artificial insemination was done. There was no difference between progesterone concentrations and pregnancy rates of treated and control group (Lucacin et al., 2010).

Heifers were synchronized with Cosynch+CIDR protocol; half of them were given double dose of flunixin meglumine (400 mg) on the 15th and 16th day after artificial insemination. On pregnancy examination, pregnancy rate was found to be 59.4% in control group and 59.5% pregnancy rate in flunixin meglumine treated group (Rabaglino et al., 2010).

The effects of flunixin meglumine on the pregnancy rates were seen on Angus heifers. In the first experiment the animals were synchronized with MGA and PGF2α. After the observation of estrus, animals were inseminated. On day 13 post artificial insemination a single dose flunixin meglumine was injected in treatment group. While pregnancy rate was 72% in control group, and it remained 66% in flunixin meglumine treated group. In the second experiment, Angus cows were synchronized via Select Synch or Select Synch + CIDR protocol. After the observation of estrus, animals were inseminated. Around 13 days after artificial insemination flunixin meglumine was injected. Pregnancy diagnosis on the 47th day revealed no difference between the control and treated group (57% vs. 58%). In third experiment both the heifers and the cows were used. The heifers were synchronized by Select Synch + CIDR protocol, and the cows were synchronized with Co-Synch + CIDR protocol. Pregnancy diagnosis was done on the 29th day; and was confirmed on the 75th day in heifers and 99th day in cows by ultrasound examination. Result showed no difference between flunixin meglumine and control group (50% vs. 48%) (Geary et al., 2010).

The carprofen and flunixin meglumine was used to see the effect on pregnancy rate in dairy cattle. The carprofen and flunixin meglumine was administered on 14th, 15th and 16th days after the insemination. Holstein- Friesian heifers (n=413) were used in the first experiment. The animals were synchronized with PGF2α they were inseminated by observing estrus. Flunixin meglumine was given at a dose rate of 2.2 mg/kg body weight to treatment group animals after on 14th-15th days or 15th -16th days of insemination. No treatment was given to animals in the control group. The pregnancy rate was found to be 58.7% in the control group and 58.6% in the treatment group. Serum progesterone levels

Review of Literature …………….
on 14th -15th days and 21st -22nd days after insemination were compared in both pregnant and non-pregnant animals. On the 21st -22nd days, progesterone levels in pregnant animals were higher. In the second experiment Holstein cows (n=380) were used and they were synchronized using ovsynch protocol. After 16 hours from the second GnRH injection, fixed time artificial insemination was done. Carprofen at a dose rate of 1.4 mg/kg was given subcutaneously on the 15th day post insemination to the treatment group. No treatment was given in control group. It was seen that the pregnancy rate in treated group was 33%, compared to 35.5% in control group (Von Kruger and Heuwiser, 2010).

In another study, cows (n= 970) were divided into three groups. In group I; carprofen was given @ 1.4 mg/kg body weight S/C following artificial insemination, in group II carprofen @ 1.4 mg/kg dose of infused into uterus 12-24 hours after the insemination and in group III no treatment was given. The pregnancy rates were 42.2%, 38.3% and 45.1%, respectively. Result showed that subcutaneous carprofen therapy had no effect on the pregnancy rate and in contrast the intrauterine therapy had a negative effect on the pregnancy rate (Heuwieser et al., 2011).

Potency of flunixin meglumine, ketoprofen and meloxicam were used in heifers. In a study performed on cows showed that meloxicam treated animals have the longest estrus cycle and meloxicam is much more potent than other NSAIDs (Amiridis et al., 2009).

In another study the meloxicam was administered in Holstein heifers at a dose of 0.5 mg/kg body weight subcutaneously on the 15th day post insemination. The pregnancy rate was 24.3% in meloxicam treated group and 52% in control group. The meloxicam application adversely affects maternal recognition of pregnancy and will be harmful to pregnancy (Rossetti et al., 2011).

Flunixin meglumine at a dose rate of 2 mg/kg body weight and oxytetracyclin 300 mg total dose, a combination was given intramuscular in cows diagnosed with subclinical endometritis. The cows were inseminated in the first estrus observed following treatment. The pregnancy rates were higher in flunixin meglumine and oxytetracyclin treated group as compared to the control group, (55%vs. 25%) (Tek et al., 2010).
The animals having puerperal metritis, ceftiofur (CEF) and flunixin meglumine were used. CEF was given to the first group for three days and in second group single dose of flunixin meglumine (2.2 mg/kg) was given intravenous in addition to CEF to the animals. They concluded that flunixin meglumine application does not have a beneficial effect on clinical recovery and reproductive performance of the animals (Drillich et al., 2007).
Materials and Methods
The present study was conducted on Cross-bred cattle at Instructional Dairy Farm (I.D.F.), Nagla of G.B. Pant University of Agriculture and Technology, Pantnagar-263145, District – Udham Singh Nagar (Uttarakhand). All the animals were kept at I.D.F. under uniform feeding and managemental conditions throughout the experiment period.

3.1 Selection of experimental cows

3.1.1 Embryo donor cows

Normal cyclic high producer cross bred cows (n=6), with age group of 3-10 years and lactation 1-5 lactations were selected on the basis of their production and reproduction history. The embryo donors had high milk producing capacity (above 18 liters peak yield/ per day), functional reproductive organs, normal reproductive cycle, absence of history of any pathological reproductive condition like pyometra, metritis, endometritis etc, on the basis of previous breeding, health records, per-rectal examination of their genital organs to reveal functional ovaries, normal uterine horns and patent cervix during diestrus. The experimental donor cows were free from diseases.

3.1.2 Embryo recipient cows

Normal cyclic cows and heifers those who have attained 250 kg body weight having functional reproductive organs, normal reproductive cycle, absence of history of any pathological reproductive condition like pyometra, metritis, endometritis etc, on the basis previous breeding, health records, per-rectal examination of their genital organs to reveal functional ovaries, normal uterine horns and patent cervix during diestrus. The experimental recipient cows were free from systemic and local diseases.

3.2 Preparation of animal

3.2.1 Deworming

Sixty days prior to starting of experiment, deworming was done with 90 ml oxytoclozanide and levamisol HCL suspension.
3.2.2 Mineral mixture feeding

Mineral mixture (Supplivite M, Zydus AH) at the dose rate of 50 gm per animal per day was started 30 days prior to experiment.

3.2.3 White side test

The white side test were performed in both donor and recipient animals during estrus to rule out sub clinical infection before experimentation. One ml estrus mucus was taken and same quantity of 5% sodium hydroxide (NaOH) solution was added in a test tube, boiled and cooled. Appearance of yellow colour was taken as evidence of infection. The infected animals were treated with intrauterine antibiotic (Lenovo-AP, Intas pharmaceuticals) for 3 days to ensure normalcy of uterine lumen.

3.3.1 Superovulation treatment during mid luteal phase of estrous cycle

The superovulation protocol was started in donor cow which came in natural heat or induced to estrus by using Cloprostenol @ 500 µg i/m (Vetmate™, Vetcare) in the cows having mature CL diagnosed by per rectal examination. All the donor animals were superovulated by using FSH-P (folltropin-V, 400 mg NIH-FSH-P1, Canada) @ 240 mg / animal as total dose given at 12 hour interval in 8 divided doses in decreasing order i.e. 45:45, 35:35, 25:25, 15:15 mg, morning and evening, by IM route starting from day 9 of estrous cycle (Day 0=day of estrus). The superestrus in donor cows were induced with IM injection of 500 µg cloprostenol with 6th dose of FSH-P (Fig. 3.1).

3.3.2 Estrus synchronization of recipient cows

Five recipient cows were prepared each per donor. Per-rectal examination was done for the presence of CL, the cows which were having CL, Cloprostenol @ 500 µg i/m (Vetmate™, Vetcare) was given on the day when donor cow came in heat i.e. on day 0 for the lysis of CL. Then all the 5 recipients were given second injection of Cloprostenol @ 500 µg i/m (Vetmate™, Vetcare) after 10 days, so that the estrus of recipient may be synchronized with superovulatory heat of donor. Recipient cows were given injection of Cloprostenol @ 500 µg i/m (Vetmate™, Vetcare) 24 hour before donor because donor was treated with FSH-P (Fig. 3.2).

3.4 Estrus detection and Artificial Insemination

After the administration of prostaglandin with the sixth dose of Folltropin V, donor cows were subjected to estrus detection. Animal were observed for sign of estrus...
Materials and Methods

Fig. 3.1: Protocol for embryo donor cow

Fig. 3.2: Protocol for embryo recipient cow

Materials and Methods
morning and evening starting from 24 hour of prostaglandin injection. Standing estrus was confirmed when treated animal stood to be mounted by other animal of the herd along with physical signs of estrus, vulvar edema, mucus discharge, per rectal examination of reproductive system and mounting on other animals. At superestrus, donor cows were bred three times at 12 hour intervals through artificial insemination using frozen semen.

3.5 Evaluation of superovulatory response

Donor cows were examined per-rectally on day 6 after superestrus (Day 0=onset of superovulatory estrus) and just prior to embryo collection to evaluate ovarian response. Both the ovaries were gently palpated and superovulated response was recorded on the basis of number of CL and unovulatory follicles. Superovulatory response was considered successful if number of CL in animal were three or more than three (Betteridge, 1980). Recipient cows were also examined per-rectum on day 6 of synchronous estrus for the presence of CL on the ovaries.

3.6 Embryo Collection

The embryos were collected non-surgically on day 7 post superovulatory estrus(day 0 = onset of superovulatory estrus) by non-surgical method using 1000 ml of flushing media per animal (Euroflush ET, IMV, France) (Image 1).

3.6.1 Laboratory preparation

All the necessary equipments, instruments, glass wares were washed properly and rinsed with double and triple distilled water and were sterilized in hot air oven at 180°C for 1 hr. Plastic wares and rubber wares (Woerlein catheter, Emcon filters, Tubing etc.) were cleaned with soap water, then with tap water followed with distilled water, double distilled and triple distilled water, then rinsed with cell culture water and dried inside incubator. Silica gel 0.2% was passed through lumen of Woerlein catheters and tubing (IMV, L’Agle, Cedex, France) were dried with the help of vacuum pump so that coating of silica gel is formed inside. All the plastic and rubber wares were sealed in polythene bags, and sterilized. Sterilization process was done a week before in advance of each embryo flushing. Towels, rubber cork for flushing bottles were sterilized in autoclave after proper cleaning and wrapping.
For embryo flushing a ready-made flushing media i.e. EUROFLUSH ET medium (IMV, Tech.) was used. Prior to embryo collection, the media bottles were maintained at 37°C by keeping the solution in the incubator.

### 3.6.2 Preparation of donor animals

The embryo donor cow was restrained in a travis. The donor was fasted overnight prior to embryo collection to reduce ruminal content. After evacuation of faeces, epidural anesthesia was induced with 3-8 ml of 2% lignocaine hydrochloride (Xylocaine®, Astra Zeneca India Ltd., Bangalore, India) in lumbo-sacral or in between 1<sup>st</sup> and 2<sup>nd</sup> coccygeal space. The perineal region and vulvar lips were thoroughly washed with antiseptic soap and water, dried with sterilized towel and disinfected with 70% alcohol. Precaution were taken to dry off alcohol prior to introduction of catheter into the vagina. The tail of the donor was tied to one side so that it is kept out of the way during flushing.

### 3.6.3 Embryo flushing

The sterilized Woerlein catheter 916/18 Gauge), tubing and embryo filter was rinsed with flushing media prior to embryo flushing. Woerlein catheter (75 cm length) fitted with sterile stylet was introduced into uterine horn to be flushed through the cervix of the animal. After smooth manipulation of catheter through cervix the inflatable balloon was placed in the uterine horn such that it is present in appropriate one third of the uterine horn near to uterine tip. The stylet was removed and balloon was inflated slowly with 12-15 ml of air according to the size of the lumen of uterine horn such that it should not damage the endometrium. Then the catheter was connected to a ‘Y’ junction of tubing, whose one end was connected to flushing medium bottle and other end to the Emcon embryo filter. After that, the flushing medium was allowed to pass into the horn lumen through the catheter. Each uterine horn was flushed 5-8 times until about 500 ml flushing medium has been used by gravitational method as described by Madan (1987). During each flushing the uterine horn was filled up with 30-60 ml of the flushing medium. The recovery was also done by the gravitational flow and passed through the embryo filter (Emcon, Immuno systems, Inc., WI, USA). During flushing, the flushed out medium from the horn, obtained in the embryo filter was so adjusted that about 15-20 ml of media always remains in the filter so as to avoid
Images 1: Embryo flushing from crossbred cow

Images 2: Embryo transfer in crossbred cow

Materials and Methods ………………
sticking of embryo in the filter. The flow of flushing media in and out of the uterine horn was controlled by tweezer clamps. After 6-7 flushing of one horn when flushed media turns out to clear i.e. no uterine contents were observed, the Woerlein catheter was withdrawn after deflation of balloon from the flushed uterine horn and again inserted into the other uterine horn and the process was repeated.

After completion of flushing process, 60 ml Lenovo-Ap was infused into the uterine horn using A.I sheath and gun to protect the animal from any possible infection and Cloprostenol Sodium (Vetmate\textsuperscript{TM}, Vetcare, 500 µg i/m ) was injected to bring the animal into estrus and to prevent implantation of embryos which could not be collected through flushing and were retained in the uterus.

After flushing, the collected filtered media was transferred to 94 x 15 mm petridish (Grenier Bio one, Germany). The embryo filter then washed with 15-20 ml of fresh flushing medium into another petridish to evaluate embryos, which might have possibly been retained in the filter’s mesh. Each petridish was searched thoroughly 3-4 times under stereozoom microscope (SMZ-2B, Nicon, Japan) at 20-40x magnification for the presence and quality of embryos recovered

### 3.7 Isolation and evaluation of embryos

The searching dishes containing embryo/ova were screened under stereozoom microscope at lower magnification (40x) 3-4 times. The embryos/ova were then searched and isolated with the help of embryo holder (Assistent Micro-Classic No-558) and transferred into small tissue culture dish (35 x 10 mm, Falcon Becton Dickinson, Labware, New Jersey, USA) containing 1.5-2.0 ml of embryo holding medium for further use. Embryos were given 2 washing in the holding medium and were evaluated under 40x magnification of stereozoom microscope for its developmental stage as well as for its quality (Image 4).

The embryos were examined, evaluated and graded morphologically as 8-16 cells, morula, blastocyst, unfertilized ova and hatched embryo using stereozoom microscope.

**8-16 cells:** The individual blastomeres were easily distinguishable with number between 8-6 cells.
**Morula:** This is commonly referred to as a “ball of cells”. Individual blastomeres are difficult to discern from one another. The cellular mass of the embryos occupies most of the perivitelline space.

**Blastocyst:** This is an embryo that has formed a fluid-filled cavity or blastocoel. Pronounced differentiation of the outer trophoblast layer are the darker, more compact inner cell mass is evident. The blastocoel is highly prominent with the embryo occupying most of the peritivelline space and at later stage it gives an appearance of signet ring. It is the best stage for transfer.

**Unfertilized:** The cytoplasmic mass of unfertilized embryo has a granular appearance without any indication of cleavage.

**Hatched embryo:** In this only crescent shape zona is seen, from which all the embryonic mass has came out.

### 3.8 Embryo transfer in synchronised recipient animals

#### 3.8.1 Embryo transfer

The transferable grade embryos were aspirated into 0.25 ml straw (IMV Technologies, L’Aigles, France). The embryo was loaded in a straw in a very unique fashion, the straw has 5 column they are (i) embryo holding medium, (ii) air, (iii) embryo holding medium + embryo, (iv) air, (v) embryo holding medium. The 5 recipient animals that were synchorinized with donor using Cloprostenol Sodium (Vetmate™, Vetcare, 500 µg i/m ) at 0 and 10th day of donor estrus, were restrained in a trevis. The feaces was evacuated and per-rectal examination was done for the presence of CL and the side of CL was also noted that on which ovary it is present. The epidural anesthesia was given, with 5-7 ml of 2% lignocaine hydrochloride (Xylocaine®, Astra Zeneca India Ltd., Bangalore, India) in lumbo-sacral or in between 1st and 2nd coccygeal space. The perineal region and vulval lips were thoroughly washed with antiseptic soap and water, dried with sterilized towel and disinfected with 70% alcohol. Precaution was taken to dry off alcohol prior to introduction of catheter into vagina. Embryos were transferred non-surgically to anterior one third of the uterine horn ipsilateral to CL using miniaturized embryo transfer gun (IMV, L’Aigle, France) covered with vaginal sheath (Image 2).
3.8.2 Experiment

Embryo transfer was done in 18 recipient cows. The cows were divided into 3 groups i.e. each group having 6 cows.

**Table 3.1: Flunixin Meglumine treatment and blood sampling schedule in recipient cows**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Blood collection</th>
<th>32 day post estrus</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (control)</td>
<td>½ hour before transfer Treatment= NSS</td>
<td>Before transfer (before treatment) (1/2 hour before treatment) and After transfer (15 min.), (50 min.) (150 min.)</td>
<td>16th - 18th day of estrous cycle (every 6 hour)</td>
</tr>
<tr>
<td>II (Treatment)</td>
<td>½ hour before transfer Treatment= flunixin meglumine @ 1.1 mg/kg body weight IM</td>
<td>Before transfer (before treatment) (1/2 hour before treatment) and After transfer (15 min.), (50 min.), (150 min.)</td>
<td>16th - 18th day of estrous cycle (every 6 hour)</td>
</tr>
<tr>
<td>III (Treatment)</td>
<td>½ hour before transfer + 16th &amp; 17th day post transfer Treatment= flunixin meglumine @ 1.1 mg/kg body weight IM</td>
<td>Before transfer (before treatment) (1/2 hour before treatment) and After transfer (15 min.), (50 min.), (150 min.)</td>
<td>16th - 18th day of estrous cycle (every 6 hour)</td>
</tr>
</tbody>
</table>

In first group, NSS was given half hour prior to embryo transfer in recipient cows and were treated as control. In second group, flunixin meglumine @ of 1.1 mg/kg b.wt was given half hour prior to embryo transfer in recipient cow. In third group, flunixin meglumine @ of 1.1 mg/kg b.wt was given half hour prior to embryo transfer in recipient cow and on 16th and 17th day post estrus, i.e. on 9th and 10th day of embryo transfer. Day 0, taken as the day of standing estrus/heat of recipient.

3.9 Blood Sampling Protocol

3.9.1 Blood sampling protocol for biochemical parameters in embryo recipient

The serum protein, glucose, cholesterol, creatinine and urea profile of crossbred recipient was analysed at different interval during embryo transfer. Before transfer i.e. on 7½ day of estrus without giving any treatment then half hour after treatment followed. Other samples were collected at 15 minute, 50 minute and 150 minute post...
transfer. On 16th, 17th and 18th day of estrus the sampling was done after every 6 hour interval and then on 32nd day. The blood collection protocol for biochemical parameter has been presented in Table 3.2.

### Table 3.2: Blood sampling protocol for biochemical parameters in embryo recipient

<table>
<thead>
<tr>
<th>Day of blood collection</th>
<th>Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day post embryo transfer</td>
<td>Before transfer</td>
</tr>
<tr>
<td></td>
<td>Before treatment</td>
</tr>
<tr>
<td>16th day</td>
<td>10 AM</td>
</tr>
<tr>
<td>17th day</td>
<td>10 AM</td>
</tr>
<tr>
<td>18th day</td>
<td>10 AM</td>
</tr>
<tr>
<td>32nd day</td>
<td>10 AM</td>
</tr>
</tbody>
</table>

#### 3.9.2: Blood sampling protocol for PGFM in embryo recipient

The serum PGFM profile of crossbred recipient was analysed at different interval during embryo transfer. Before transfer i.e. on 7½ day of estrus without giving any treatment then half hour after treatment followed. Other samples were collected at 15 minute, 50 minute and 150 minute post transfer. On 16th, 17th and 18th day of estrus the analyses was done after every 12 hour interval and then on 32nd day. The blood collection protocol for PGFM has been presented in Table 3.3.

### Table 3.3: Blood sampling protocol for PGFM in embryo recipient

<table>
<thead>
<tr>
<th>Day of blood collection</th>
<th>Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day post embryo transfer</td>
<td>Before transfer</td>
</tr>
<tr>
<td></td>
<td>Before treatment</td>
</tr>
<tr>
<td>16th day</td>
<td>10 AM</td>
</tr>
<tr>
<td>17th day</td>
<td>10 AM</td>
</tr>
<tr>
<td>18th day</td>
<td>10 AM</td>
</tr>
</tbody>
</table>
3.9.3: Blood sampling protocol for progesterone in embryo recipient

The serum PGFM profile of crossbred recipient was analysed at different interval during embryo transfer. Before transfer i.e. on 7½ day of estrus without giving any treatment followed by 150 minute post transfer. On 16\textsuperscript{th}, 17\textsuperscript{th} and 18\textsuperscript{th} day of estrus one sample was taken and then on 32\textsuperscript{nd} day. The blood collection protocol for progesterone has been presented in Table 3.4.

Table 3.4: Blood sampling protocol for progesterone in embryo recipient

<table>
<thead>
<tr>
<th>Day of blood collection</th>
<th>Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day post embryo transfer</td>
<td>Before transfer</td>
</tr>
<tr>
<td></td>
<td>After transfer</td>
</tr>
<tr>
<td></td>
<td>Before treatment</td>
</tr>
<tr>
<td></td>
<td>150 minutes</td>
</tr>
<tr>
<td>16\textsuperscript{th} day</td>
<td>10 PM</td>
</tr>
<tr>
<td>17\textsuperscript{th} day</td>
<td>10 PM</td>
</tr>
<tr>
<td>18\textsuperscript{th} day</td>
<td>10 PM</td>
</tr>
<tr>
<td>32\textsuperscript{nd} day</td>
<td>10 AM</td>
</tr>
</tbody>
</table>

3.10 Collection of blood sample

Blood samples (10-12 ml) were collected as per schedule (Table 3.2, 3.3 and 3.4) in sterilized test tube and kept at room temperature as a slant for about 3-4 hour for separation of blood serum. Blood serum was separated, using centrifuge machine at 3000 rpm for 15 minutes and was transferred into sterilized serum vials and stored at -20\degree C till analysis in deep freezer.

3.11 Ultrasonographic Examination

Monitoring of follicular development, superovulatory response was performed by B-mode real time ultrasonographic (DIGI 600, PRO VET, SS medical, India) scanning of ovaries on 21\textsuperscript{th} days of estrous cycle for the number of CL formed on both the ovaries and were recorded (Image 3).

3.12 Pregnancy diagnosis

The examination of pregnancy was done in cows on 45\textsuperscript{th} day of embryo transfer.

Materials and Methods ……………..
3.13. Estimation of biochemical parameters

Biochemical parameters; Glucose, Cholesterol, Total Protein, Urea and Creatinine was estimated in serum samples of recipient cow using diagnostic kits following standard protocol.

3.13.1 Total protein

The plasma total protein in blood serum was determined by using diagnostic kit manufactured by SPAN diagnostics Ltd., Surat, India.

**Principle**: The peptide bond of protein react with cupric ions in alkaline solution to form a coloured chelate, the absorbance of which was measured at 578 nm. One ml of reconstituted reagent was mixed with 10 µl plasma and the absorbance was recorded at 578 nm wavelength in spectrophotometer. Simultaneously 10 µl of standard (Bovine serum albumin) was mixed with 1 ml of reconstituted reagent and absorbance was recorded after 5 minutes of incubation.

<table>
<thead>
<tr>
<th>Pipette into tubes marked</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>1000 µl</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Distilled water</td>
<td>20 µl</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>20 µl</td>
<td>-</td>
</tr>
<tr>
<td>Test</td>
<td>-</td>
<td>-</td>
<td>20 µl</td>
</tr>
</tbody>
</table>

Calculation:

\[
\text{Total protein (g/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 6g/dL
\]

3.13.2 Glucose

Was estimated by Glucose Test Kit (GOD-POD method) manufactured by ARKRAY Healthcare Pvt. Ltd. (Surat) India.

**Principle**: Glucose oxidase oxidises Glucose to Gluconic Acid and Hydrogen peroxide. In presence of enzyme Peroxidase, released Hydrogen peroxide is coupled with phenol and 4-Aminoantipyrine to form coloured Quinonemine dye. 10 µl of sample was mixed
Images 3: Examination of superovulatory response using ultrasound
with working glucose reagent, and was incubated at 37 °C for 10 minutes. Absorbance of coloured dye is measured at 505 nm and is directly proportional to Glucose concentration in the sample.

<table>
<thead>
<tr>
<th>Pipette into tubes marked</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum / Plasma</td>
<td>-</td>
<td>-</td>
<td>10 µl</td>
</tr>
<tr>
<td>Reagent 3</td>
<td>-</td>
<td>10 µl</td>
<td>-</td>
</tr>
<tr>
<td>Working Glucose Reagent</td>
<td>1000 µl</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
</tbody>
</table>

Calculation:

\[
\text{Glucose (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 100
\]

3.13.3 Cholesterol

Was estimated by Cholesterol Test Kit manufactured by ARKRAY Healthcare Pvt. Ltd. (Surat) India.

Principle: Cholesterol ester hydrolysed by Cholesterol Estease to give free Cholesterol and Fatty acid. In subsequent reaction, Cholesterol Oxidase oxidises the 3-OH group of free Cholesterol to liberate Cholest-4-en-3-one and Hydrogen Peroxide. In presence of Peroxidase, Hydrogen Peroxide couples with 4-Aminoantipyrine and phenol to produce Red Quinonemine dye. One ml working reagent was mixed with 10 µl of sample, incubated for 10 minute at room temperature (15-25 °C) and the absorbance of coloured dye was measured at 505 nm and is proportional to amount of Total Cholesterol concentration in the sample.

<table>
<thead>
<tr>
<th>Pipette into tubes marked</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum / Plasma</td>
<td>-</td>
<td>-</td>
<td>10 µl</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>-</td>
<td>10 µl</td>
<td>-</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>1000 µl</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
</tbody>
</table>

Materials and Methods ...............
Calculation:

\[
\text{Cholesterol concentration (mg/dl) } = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200
\]

3.13.4 Creatinine

Was estimated by Alkaline Picate Method using creatinine diagnostic Test Kit manufactured by Coral Clinical System (Goa) India.

**Principle:** Picric acid in an alkaline medium reacts with creatinine to form an orange complex with the alkaline picate. Intensity of the colour formed is directly proportional to the amount of creatinine present in the sample. The deproteinization of specimen is done by adding Picric acid reagent (L1) 2.0 ml to 0.2 ml of sample, mixed well and centrifuged at 2500-3000 rpm for 10 minutes to obtain clear supernatant. The absorbance of the standard and Test sample were taken against Blank at 520 nm.

<table>
<thead>
<tr>
<th>Additional sequence</th>
<th>Blank (ml)</th>
<th>Standard (ml)</th>
<th>Test (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>1.1</td>
</tr>
<tr>
<td>Picric Acid Reagent(L1)</td>
<td>1.0</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>Distilled water</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Creatinine Standard (S)</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>Buffer reagent (L2)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Calculation:

\[
\text{Creatinine in mg\% } = \frac{\text{Absorbance of the Test Sample}}{\text{Absorbance of the Standard}} \times 2.0
\]

3.13.5 Urea

Was estimated by Berthelot Method using Urea diagnostic Test Kit manufactured by BEACON Diagnostics Pvt. Ltd. (Navsari) India.

**Principle:** Urease breaks down urea into ammonia and carbon dioxide in alkaline medium. Ammonia liberated from the breakdown of Urea reacts with Hypochloride
and salicylate to form dicarboxyindophenol. This reaction is catalysed by the presence of Nitroprusside. The intensity of the colour produced by the reaction is directly proportional to the concentration of urea present in the sample. 10 µl of sample was mixed with 2 ml of reagent mixed well and incubated at 37 °C for 5 minutes and it is measured photometrically at 600 nm within 60 minutes.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent 1</td>
<td>1 ml</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>10 µl</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>10 µl</td>
</tr>
</tbody>
</table>

Mix well and Incubate at 37°C for 5 min.

| Reagent 2 |         | 1 ml     | 1 ml     | 1 ml     |

Calculation:

\[
\text{Urea Concentration} = \frac{\text{O.D. Sample}}{\text{O.D. Standard}} \times 40
\]

3.14 Hormonal Estimation

Progesterone and PGFM were estimated in blood serum by radioimmunoassay (RIA) using RIA kits and ELISA respectively.

3.14.1 Prostaglandin Fetal metabolite (PGFM) Estimation:

The estimation of PGFM was done by Bovine Prostaglandin FM (PGFM) ELISA Kit Bioassay Technology Laboratory, Shanghai Korain Biotech CO., LTD., Junjiang, China.

\[
\text{CV}(\%) = \frac{\text{SD}}{\text{mean}} \times 100; \text{Intra-Assay: CV}< 10\%; \text{Inter-Assay: CV}< 12\%
\]

Serum which was kept at -20 °C was thawed for 10-20 minutes at room temperature. Centrifuged (at 2000-3000 RPM) for 20 minutes.

Assay procedure

1. Dilution of standard solutions:

Kit had a standard of original concentration, which was diluted in small tubes by using independently following the instruction.
<table>
<thead>
<tr>
<th>Concentration</th>
<th>Standard No.</th>
<th>Diluent Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>480ng/L</td>
<td>5</td>
<td>120µl Original Standard + 120µl Standard diluents</td>
</tr>
<tr>
<td>240ng/L</td>
<td>4</td>
<td>120µl Standard No.5 + 120µl Standard diluents</td>
</tr>
<tr>
<td>120ng/L</td>
<td>3</td>
<td>120µl Standard No.4 + 120µl Standard diluents</td>
</tr>
<tr>
<td>60ng/L</td>
<td>2</td>
<td>120µl Standard No.3 + 120µl Standard diluents</td>
</tr>
<tr>
<td>30ng/L</td>
<td>1</td>
<td>120µl Standard No.2 + 120µl Standard diluents</td>
</tr>
</tbody>
</table>

2. The number of stripes needed was determined by number of samples to be tested added by that of standards. It was suggested that each standard solution and each blank well should be arranged with three or more wells (Image 5).

3. Sample injection:
   a) Blank well: no sample, anti PGFM antibody labeled with biotin or streptavidin-HRP was added to comparison blank well except chromogen solution A & B and stop solution while taking the same steps that follow.
   b) Standard solution well: 50µl standard was added and streptavidin-HRP 50µl (biotin antibodies had united in advance in the standard so no biotin antibodies were added.)
   c) Sample well to be tested: 40µl sample was added and then 10µl PGFM antibodies, 50µl streptavidin-HRP. Then it was covered with seal plate membrane and was gently shaken to mix them up and incubated at 37°C for 60 minutes.

4. Preparation of washing solution: Washing concentration (30X) was diluted with distilled water for later use.

5. Washing: Sealed plate membrane was removed carefully, the liquid was drained and the remaining liquid was shooked off. Each well was filled with washing solution. Then the liquid was drained after 30 seconds’ standing and this procedure was repeated five times and plate was bloted.

6. Color development: 50µl of chromogen solution A was added, firstly to each well and then added 50µl chromogen solution B to each well. Then the plate was Shaken gently to mix them up and was incubated for 10 minutes at 37°C away from light for colour development (Image 6).
Images 4: Evaluation of embryo using stereozoom microscope

Images 5: Estimation of PGFM in blood serum using ELISA
Images 6: ELISA plate before adding stop solution

Images 7: ELISA plate after adding stop solution
7. Stop: 50µl Stop Solution was added to each well to stop the reaction (the blue colour changes into yellow immediately at that moment) (Image 7).

8. Assay: The blank well was taken as zero, absorbance (OD) of each well was measured one by one under 450nm wavelength, which was carried out within 10 minutes after adding the stop solution.

9. According to standards’ concentrations and the corresponding OD values, was calculated and the linear regression equation of the standard curve. Then according to the OD value of samples, concentration of the corresponding sample was calculated.

**Calculation:**

The standard curve was drawn on the coordinate paper. According to the OD value of the sample, corresponding concentration was located (which is the concentration of the sample); or calculated) the linear regression equation of standard curve according to the concentration of the standard and the OD value. Then substitute with the OD value of the sample to calculate its concentration.

Assay range : 3ng/L → 900ng/L.; Sensitivity : 1.56ng/L.

**SUMMARY**

Prepare reagents, samples and standards.

Add prepared samples and standards together with second antibody labeled with biotin and ELISA solutions. Let them react for 60 minutes at 37℃.

Wash the plate five times. Add Chromogen solution A and B. Incubate for 10 minutes at 37 ℃ for color development.

Add stop solution.

Read the OD value within 10 minutes.

Calculate.
3.14.2 Progesterone Estimation

The estimation of progesterone in samples was done by using Progesterone C.T. RIA kit (M/ S Beckman Coulter IM 1188) at IVRI (Nuclear Research Laboratory under Division of Physiology and Climatology).

i. Principle of assay of plasma progesterone

The radioimmunoassay of plasma progesterone is a competitive assay. Samples and standards with \(^{125}\text{I}\)-labeled plasma progesterone, as traces, in antibody coated tubes for plasma progesterone estimation, after incubation, the content of the tubes were aspirated to remove the unbound material and then, bound radioactivity was measured. The amount of labelled bound to solid-phase antibody is inversely related to the amount of unlabeled hormones present in the standard or samples. A calibration curve was established and unknown values were determined by interpolation from the curve.

ii. Analytical sensitivity: 0.03 ng/ mg (0.10 nmol/L).

iii. Specificity: The antibody used in the immunoassay is highly specific for progesterone.

iv. Assay procedure of plasma progesterone

All the reagents were brought to room temperature (20-25°C) before assaying. Reagent were dispensed in the bottom of the antibody coated tubes and procedure operated according to the following.

1. For the estimation of plasma progesterone, 50 µl standard and 50 µl standard plasma sample were taken into antibody coated tubes.

2. 500 µl \(^{125}\text{I}\)-labelled plasma progesterone was added to each antibody- coated tubes.

3. Two additional non-coated tubes were prepared for the total activity computation containing 500 µl tracer (\(^{125}\text{I}\)-labelled plasma progesterone) and was set aside until counting.

4. The contents of the tubes were mixed with a vortex and incubated for 1 hour for plasma progesterone estimation at room temperature, while continuously shaking (300-350 rpm).
5. The incubation mixture was carefully aspirated with aspirator tip touched the bottom of the antibody coated tube so that all the liquid was removed.

6. The radioactivity was measured with the help of SR-300 fully automatic Gamma Counter.

3.15 Statistical analysis

The data obtained during the study was analyzed for mean, standard error in mean and coefficient of correlation. Difference between means was compared using two sample t-test and ANOVA (Snedecor and Cochran, 1994) by completely randomized design (CRD); for storage stability studies Randomized Block Design (RBD) was followed.
Results and Discussion
Superovulation and embryo transfer is an efficient tool for producing large number of high quality seed stock and to maximize the number of young born from females of high genetic quality. The present study was designed to evaluate the effect of flunixin meglumine on embryo recipient cattle with the hypothesis that prostaglandin released during uterine manipulation before and during embryo transfer which is detrimental to normal embryonic development would be reduced. Flunixin meglumine, a COX inhibitor will help in reducing \( \text{PGF}_2 \) during critical development of embryo. Thus, may be helpful in improving conception following embryo transfer cattle. Eighteen animals were taken as embryo recipient and divided into three groups i.e. control (n=6), treatment group first (T-I, n=6) and treatment group second (T-II, n=6).

In group I no treatment was given and kept as control, in T-I flunixin meglumine was given @ 1.1 mg/kg body weight half an hour before embryo transfer and in T-II flunixin meglumine was given ½ hour before embryo transfer and on 16\(^{th}\) and 17\(^{th}\) day of estrous cycle. So that the pulsatile release of prostaglandin is minimized during luteal phase.

The results obtained are discussed under following heads:

1. Superovulatory response and embryo recovery
2. Embryonic development and quality
3. Biochemical profile
   3.1 Total serum protein
   3.2 Total serum glucose
   3.3 Total serum cholesterol
   3.4 Total serum creatinine
   3.5 Total serum urea
4. Hormonal profile
   4.1 Prostaglandin fetal metabolite (PGFM)
   4.2 Progesterone
5. Conception following transfer of crossbred embryos.
6. The effect of flunixin meglumine on PGFM and progesterone level in Control non-pregnant, Treated non-pregnant and Treated pregnant/expected pregnancy.
1. **Superovulatory response and embryo recovery**

A total of ten donor (Crossbred) cows were subjected to superovulation treatment. The response of donor cows to superovulation treatment and corresponding embryo recovery profile is given in Table 4.1.

Nine of the ten cows responded (≥ 3) to the superovulatory treatment presented in Table 4.2 and Fig 4.1. A total of 96 ovulation were counted by per-rectal palpation and ultrasonography on both the ovaries. The mean ovulation rate (number of CL) was 9.6±1.84. The total recovered embryos/ova were 63. The mean embryo/ova recovered per donor and recovery rate was 6.3±1.84 and 65.62% respectively are depicted in Table 4.3 and Fig 4.2.

The overall superovulatory response was 90% which is similar to that reported by Rawat (2004) i.e 89.47%, 85.7% Ferreira et al. (2014) but it was higher than 76.5% (Betteridge et al., 1980). One cow does not respond to FSH treatment and no palpable CL was found. Baruselli et al. (2006) observed that 20-30% donors were unresponsive to superstimulation. Previous studies have concluded that usually 33% animals did not respond to superovulatory regimen even after following standard superovulatory protocol (Misra, 2002; Misra and Pant, 2006). Several workers have also found that a large proportion of animals failed to respond to superovulatory treatment which could be considered as normal (Mittal et al., 1999; Kasiraj et al., 2000).

The result of the superovulatory treatment used in the present experiment indicate that there were more number of CL. Mean ovulation rate (3-7 CL) in this study was higher in accordance with report of Beg et al.(1996). However, overall ovulation rate of 9.6±1.75 was nearly similar to that 9.5 CL (Mikkola and Taponen, 2017), 9.2±3.8 CL (Baruselli et al., 2000) and 8.8 CL per cow (Karaivanov et al.1990), it was higher than 7.1±4.44 CL (Posadas et al., 1991), 6.8 CL (Situmorang, 2005), 5.4±1.4 CL in heifers (Vos et al., 1994), 3.58±0.49 CL (Heleil et al., 2010) and 3.6 – 4.3 CL (Drost et al., 1988) but lower than the number of corpus lutea found in Nguni cows 11.3±1.41 (Maqhashu et al., 2015), 16.1 CL per cow (Hay et al., 1990), 25±9.2 CL and the number of ovulation was within the range reported by Rajamahendran et al. (1976) i.e. 1 to 17 number of corpora lutea (CL) in the heifers, 4 – 37 CL in cows (Roberts and Echternak,1993) and 7–32 CL in Holstein heifers following follitropin treatment (Carvalho et al., 2014).
Table 4.1: Superovulatory response and embryo recovery in folltropin-V treated crossbred cows.

<table>
<thead>
<tr>
<th>Cow no.</th>
<th>CL</th>
<th>TE</th>
<th>UFO</th>
<th>DEG</th>
<th>4-16 cell</th>
<th>M</th>
<th>Bl</th>
<th>Zona</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>17</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>16</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>11</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>11</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>96</td>
<td>63</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>22</td>
<td>36</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 4.2: Superovulatory response in embryo donor cow following folltropin-V treatment (n=10).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>No. cows</th>
<th>Attributes</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>Animal responded</td>
<td>90%</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Animal does not responded</td>
<td>10%</td>
</tr>
</tbody>
</table>

Table 4.3: Embryo/ova recovery rate in embryo donor cow following folltropin-V treatment (n=10).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Attributes</th>
<th>Number</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Number of C.L</td>
<td>96</td>
<td>9.6±1.84</td>
</tr>
<tr>
<td>2</td>
<td>No of recovery</td>
<td>63</td>
<td>6.3±1.84</td>
</tr>
</tbody>
</table>
Fig. 4.1: Superovulatory response in embryo donor cow following folltropin-V treatment (n=10).

Fig. 4.2: Embryo ova/recovery rate in embryo donor cow following folltropin-V treatment (n=10).

The mean ovulation rate was similar to the 63.9±8.6% embryos/ova recovered (Sartori et al., 2003), recovery rate 69.7% and embryo recovered per cow 6.62 (Acosta et al., 2016), 6.2±4.7 embryos/ova (Posadas et al., 1991), 6.7 embryos (Ferreira et al., 2014), 6.4 embryos (Hay et al., 1990), the embryos/ova recovery was higher than 5.4±0.8 (Kohram et al., 1998), 4.8 embryos (Betteridge et al., 1980), 4.7±1.7 embryos (Subramaniam et al., 1991), 39.27 per cent (Sharma, 2004) and Prasad (2000) who reported total embryo recovery rate of 32.40 per cent, but lower than 8.4 recovered embryos (Junior et al., 2008), 8.3±0.4 embryos (Neto et al., 2005).
In one animal only one embryo could be recovered with recovery rate of 14.28%. The lower embryo recovery rate in relation to the number of ovulation may be due to inability of fimbria to trap ova from enlarged superovulatory ovary (Ullah et al., 1992), difficulties in locating hatched blastocyst (Alexiev et al., 1988) and premature entry of ova/embryo into the uterus, resulting into their expulsion (Karaivanov et al., 1990).

2. Embryonic development and quality

A total 63 embryo/ova were recovered from donor cow, the mean total and average were 6.3±1.84 and 65.25 respectively embryos/ova per animal per flush. A total of 58 good quality of transferable embryos were recovered with mean number of 5.8±1.81. In present study, the stages of embryonic development were also evaluated by dividing them in 6 groups i.e. unfertilized, 4-16 cells (Image 8), morula (Image 9), blastocyst (Image 10, 11, 12, 13 and 14), degenerated (Image 17) and hatching embryo/empty zona (Image 15 and 16). A total number of 4-16 cell stage, morula, blastocyst, unfertilized, degenerated and empty zona recovered were 2, 22, 36, 1, 1 and 1 respectively, on pooling the data mean total 4-16 cell stage, morula, blastocyst, unfertilized, degenerated and empty zona recovered were 0.2±0.20, 2.2±0.57, 3.6±1.36, 0.1±0.10, 0.1±0.10 and 0.1±0.10 respectively. The average recovery of 4-16 cell stage, morula, blastocyst, unfertilized, degenerated and empty zona were 3.17%, 34.92%, 57.14%, 1.58%, 1.58% and 1.58% respectively are presented in Table 4.4 and Fig 4.3.

The recovery of blastocyst and morula was similar to that of Shaw and Good (1999) who reported that the recovery of grade I, grade II and grade III were 3.6±0.1, 1.5±0.1 and 1.6±0.1 respectively, but higher mean total recovery of fertile dead and nonfertile ova 2.4±0.1 and 4.1±0.2 respectively. The total recovery, unfertilized embryo and degenerated embryo were 6.20, 2.53 and 0.32 respectively that is similar to the above finding but degenerated embryo and congested embryo were lower 40.8% and 5.16% respectively (Albuquerque et al., 2012). The stages of embryo recovered from Nguni cow code I and code II, 2.5±1.0 and 1.3±0.59 respectively were slightly similar whereas unfertilized ova and degenerated embryo 5.5±1.05 and 3.7±1.00 respectively were lower (Maqhashu et al., 2015). The mean number of total embryos/ova recovered 5.16±2.58 were nearly similar, but the degenerated embryo and unfertilized ova recovered were higher 2.50±2.30 and 1.66±1.47 respectively in cattle (Sharma, 2004). Mikkola and Taponen (2017) reported that UFO and degenerated embryo recovered
were 2.9 and 2.0 respectively following Folltropin treatment in dairy cow which is higher than the present study.

The mean total good quality embryos were similar to 58.3% reported by Critser et al. (1980), 5.7±0.2, 6.5±1.7 and 7.1 good quality transferable embryos (Shaw and Good 1999), (Carvalho et al., 2014) and (Mikkola and Taponen 2017) respectively, nearly similar to 4.5±3.9 good quality embryos (Takahashi et al., 2013) and it was higher than mean total 1.37±0.43 viable embryos (Rawat, 2004) and were more than the transferable embryos 2.8 and 1.00±0.63 found following embryo recovery (Ferreira et al., 2014) and (Sharma, 2004) respectively, but lower than average transferable embryo 74.90% (Schiew et al., 1987) and Dattena et al. (1994) who reported that 77% of good quality embryos recovered following tapering dose of pFSH in ewes.

Totey et al. (1988) reported that high doses of FSH can also cause hypersecretion of progesterone 2-4 days post estrus. Embryos are normally in oviduct during this period and do not enter the uterus until day 4. However, abnormally high level of progesterone may eventually force the embryos prematurely into the uterus. The uterine environment, being hostile to embryo prior to day 4, may halt cleavage and causes degeneration of embryo.

Table 4.4: Stages of embryo recovered from embryo donor cow following folltropin-V treatment (n=10).

<table>
<thead>
<tr>
<th>S.No</th>
<th>Embryo stages</th>
<th>Number</th>
<th>Mean</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Unfertilized</td>
<td>1</td>
<td>0.1±0.10</td>
<td>1.58%</td>
</tr>
<tr>
<td>2</td>
<td>Blastocyst</td>
<td>36</td>
<td>3.6±1.36</td>
<td>57.14%</td>
</tr>
<tr>
<td>3</td>
<td>Morula</td>
<td>22</td>
<td>2.2±0.57</td>
<td>34.92%</td>
</tr>
<tr>
<td>4</td>
<td>4-16 cell</td>
<td>2</td>
<td>0.2±0.20</td>
<td>3.17%</td>
</tr>
<tr>
<td>5</td>
<td>Empty zona</td>
<td>1</td>
<td>0.1±0.10</td>
<td>1.58%</td>
</tr>
<tr>
<td>6</td>
<td>Degenerated</td>
<td>1</td>
<td>0.10±0.10</td>
<td>1.58%</td>
</tr>
<tr>
<td>7</td>
<td>Total embryo/ova</td>
<td>63</td>
<td>6.3±1.84</td>
<td></td>
</tr>
</tbody>
</table>
Results and Discussion

Images 8: 4 cell stage embryo 4x magnification

Images 9: Morula 4x magnification
Images 10: Blastocyst at 4x magnification

Images 11: Blastocyst at 10x magnification

Results and Discussion ……………
Images 12: Blastocyst at 10x magnification

Images 13: Blastocyst at 20x magnification
Results and Discussion

Images 14: Blastocyst at 40 x magnification

Images 15: Hatching blastocyst at 20x magnification
Images 16: Hatched zona 4x magnification

Images 17: Degenerated embryo at 4x magnification
Fig. 4.3: Stages of embryo recovered from embryo donor cow following folltropin-V treatment (n=10).

3. Biochemical profile of recipient cows

The various biochemical parameters were analysed before and after flunixin meglumine treatment.

3.1 Total Serum Protein

The mean serum protein concentration in Control, T-I and T-II group before transfer at zero hour and half hour after treatment was 6.52±0.09, 6.39±0.06 and 6.44±0.06 and 6.55±0.09, 6.36±0.06 and 6.42±0.06 g/dl respectively. There was no significant difference between the groups.

The mean serum protein concentration at 15 minutes, 50 minutes and 150 minutes after transfer in Control, T-I and T-II group was 6.53±0.09, 6.36±0.05 and 6.39±0.04 and 6.46±0.06, 6.35±0.04 and 6.41±0.06 and 6.40±0.06, 6.35±0.05 and 6.38±0.05 g/dl respectively and there was no significant difference between the groups (Fig 4.4).
The mean protein concentration on 16\textsuperscript{th}, 17\textsuperscript{th} and 18\textsuperscript{th} day of estrus at 0 (zero) hour, 6 hour, 12 hour and 18 hour in Control, T-I and T-II was non-significant difference between the group. On 32\textsuperscript{nd} day the mean protein concentration in Control, T-I and T-II was 6.44±0.06, 6.74±0.12 and 6.50±0.10 g/dl respectively and there was no significant difference between the groups. The mean serum protein concentration remained same between treated and control groups (Table 4.5 and Fig 4.5).

There was a slight decrease in the mean protein level in T-I and T-II group as compared to control after administration of flunixin meglumine on the day of embryo transfer. In T-II there is slight decrease in protein concentration on day 16\textsuperscript{th}, 17\textsuperscript{th} and 18\textsuperscript{th} at different hours after injecting flunixin meglumine as compared to T-I and Control. The highest mean concentration of protein was recorded in T-I (6.74±0.12 g/dl) than Control (6.55±0.09 g/dl) and then T-II (6.50±0.10 g/dl).

The mean concentration of total serum protein observed in the present study as similar with the reports of Memon (1961), Takahashi \textit{et al.} (2013), Pandey \textit{et al.} (2016), and Sheetal (2017) but lower than Kumar (2014) and Ahmad \textit{et al.} (2004). Similar values of mean total protein concentration were reported by Abd Ellah \textit{et al.} (2010) during different stages of estrous cycle in buffaloes. Serum total protein concentration does not differ significantly following flunixin meglumine treatment was in agreement with the findings of Safarchi \textit{et al.} (2010) in which they gave flunixin meglumine repeatedly at 12 hour interval for 12 days in crossbred goats. Carrick \textit{et al.} (1989) used Flunixin Meglumine at the dose rate of 1.1 mg/kg body weight in foal and found no significant difference but decrease in protein level. Erdogan \textit{et al.} (2003) who used Flunixin Meglumine at the dose rate of 1.1 mg/kg body weight in dogs for 5 days intravenously was not consistent with the present findings. The total protein level in pregnant and non-pregnant did not differ significantly (p>0.05) in Control, Buserlin acetate, hCG and TRIU-B Groups in repeat breeder cows on different days of estrus cycle i.e. 0, 5, 10, 15 and day 20 (Pandey \textit{et al.}, 2016).

There was no direct effect on protein level using flunixin meglumine. The decrease in protein level is due to Gastroenteropathy which causes loss of protein when NSAIDs are used repeatedly (Safarchi \textit{et al.}, 2010).
Table 4.5: Mean (±SE) serum Protein concentration (g/dl) of different groups on day of transfer (day 7), 16\textsuperscript{th}, 17\textsuperscript{th}, 18\textsuperscript{th} and 32\textsuperscript{nd} day post estrus in crossbred recipients.

<table>
<thead>
<tr>
<th>Time</th>
<th>Control (g/dl)</th>
<th>T-I (g/dl)</th>
<th>T-II (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before transfer</td>
<td>Before treatment</td>
<td>0 hr</td>
<td>6.52±0.09</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>1/2 hr</td>
<td>6.55±0.09</td>
</tr>
<tr>
<td>After transfer</td>
<td>Day of transfer</td>
<td>15 min</td>
<td>6.53±0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 min</td>
<td>6.46±0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150 min</td>
<td>6.40±0.06</td>
</tr>
<tr>
<td>16\textsuperscript{th} day</td>
<td></td>
<td>0 hr</td>
<td>6.39±0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 hr</td>
<td>6.35±0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 hr</td>
<td>6.51±0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18 hr</td>
<td>6.18±0.08</td>
</tr>
<tr>
<td>17\textsuperscript{th} day</td>
<td></td>
<td>0 hr</td>
<td>6.43±0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 hr</td>
<td>6.44±0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 hr</td>
<td>6.30±0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18 hr</td>
<td>6.40±0.06</td>
</tr>
<tr>
<td>18\textsuperscript{th} day</td>
<td></td>
<td>0 hr</td>
<td>6.39±0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 hr</td>
<td>6.29±0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 hr</td>
<td>6.54±0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18 hr</td>
<td>6.39±0.05</td>
</tr>
<tr>
<td>32\textsuperscript{nd} day</td>
<td></td>
<td>6.44±0.06</td>
<td>6.74±0.12</td>
</tr>
</tbody>
</table>

Mean differed non-significantly (p> 0.05) between the groups.
Fig. 4.4: Mean (±SE) serum protein concentration (g/dl) of different groups on day of embryo transfer (day 7) at different intervals in crossbred embryo recipient.

Fig. 4.5: Mean (±SE) serum protein concentration (g/dl) of different groups on 16th, 17th, 18th and 32nd day of estrus in crossbred embryo recipient.
3.2 Total Serum Glucose

The mean serum glucose concentration in Control, T-I and T-II group before transfer at zero hour and half hour after treatment was 55.44±1.06, 56.29±1.17 and 58.95±4.27 and 56.96±2.79, 57.59±1.49 and 57.30±1.20 mg/dl respectively. There was no significant difference between the groups.

The mean serum glucose concentration at 15 minutes, 50 minutes and 150 minutes after transfer in Control, T-I and T-II group was 55.49±1.16, 58.57±1.97 and 57.85±1.00 and 58.40±3.72, 59.37±1.97 and 56.41±1.63 and 60.17±3.15, 59.45±1.74 and 58.70±2.13 mg/dl respectively and there was no significant difference between the groups (Fig 4.6).

The mean glucose concentration on 16th, 17th and 18th day of estrus at 0 (zero) hour, 6 hour, 12 hour and 18 hour in Control, T-I and T-II was non-significantly difference between the group. On 32nd day the mean glucose concentration in Control, T-I and T-II was 60.00±2.15, 60.51±1.40 and 58.35±0.59 mg/dl respectively and differed non-significantly between the groups. The mean serum glucose concentration remained same between treated and control groups. The highest mean concentration of glucose was recorded in T-I (62.91±1.40 mg/dl) than T-II (60.72±1.29 mg/dl) and then Control (60.17±3.15 mg/dl) (Table 4.6 and Fig 4.7).

The mean serum concentration reported in the present study was similar to that of (Kappel et al., 1984; Lager et al., 2012; Sreedhar et al., 2013). Erdogan et al. (2003) who used flunixin meglumine at the dose rate of 1.1 mg/kg body weight in dogs for 5 days intravenously and observed no significant changes in the blood glucose concentration but there was an increase in blood glucose level after using dexamethasone and prednisolone in normal cows (Maplesden et al., 1960).

Circulating glucose concentration were lower in sever negative energy balance (SNEB) compared to mild negative energy balance (MNEB) in lactating dairy cows (Fenwick et al., 2008) and in mares (Salazar-ortiz et al., 2014). These variations in blood glucose level might occur due to feeding of imbalance ration to dairy cows. There is decrease in blood glucose concentration in cows with advancement of pregnancy in winters (Alameen and Abdelatif, 2012). Glucose is also essential for gonads to maintain the quality of oocytes, development of embryo (Sutton-McDowall et al., 2010; Rato et al., 2012).
Table 4.6: Mean (±SE) serum glucose concentration (mg/dl) of different groups on day of transfer (day 7), 16<sup>th</sup>, 17<sup>th</sup>, 18<sup>th</sup> and 32<sup>nd</sup> day post estrus in crossbred recipients.

<table>
<thead>
<tr>
<th></th>
<th>Glucose (mg/dl)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>Before transfer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>0 hr</td>
<td>55.44±1.06</td>
<td>56.29±1.17</td>
</tr>
<tr>
<td>After treatment</td>
<td>1/2 hr</td>
<td>56.96±2.79</td>
<td>57.59±1.49</td>
</tr>
<tr>
<td>After transfer</td>
<td>Day of transfer</td>
<td>15 min</td>
<td>55.49±1.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 min</td>
<td>58.40±3.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150 min</td>
<td>60.17±3.15</td>
</tr>
<tr>
<td>16&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>0 hr</td>
<td>55.70±1.57</td>
<td>58.31±2.04</td>
</tr>
<tr>
<td></td>
<td>6 hr</td>
<td>57.26±1.57</td>
<td>59.32±1.28</td>
</tr>
<tr>
<td></td>
<td>12 hr</td>
<td>55.82±1.52</td>
<td>56.88±1.49</td>
</tr>
<tr>
<td></td>
<td>18 hr</td>
<td>59.96±1.25</td>
<td>61.14±2.28</td>
</tr>
<tr>
<td>17&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>0 hr</td>
<td>56.92±2.05</td>
<td>59.16±1.72</td>
</tr>
<tr>
<td></td>
<td>6 hr</td>
<td>57.26±1.48</td>
<td>60.46±1.90</td>
</tr>
<tr>
<td></td>
<td>12 hr</td>
<td>57.68±2.16</td>
<td>62.11±1.58</td>
</tr>
<tr>
<td></td>
<td>18 hr</td>
<td>57.05±1.38</td>
<td>61.40±1.60</td>
</tr>
<tr>
<td>18&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>0 hr</td>
<td>57.51±1.81</td>
<td>61.12±1.64</td>
</tr>
<tr>
<td></td>
<td>6 hr</td>
<td>55.86±1.80</td>
<td>61.35±1.83</td>
</tr>
<tr>
<td></td>
<td>12 hr</td>
<td>55.61±1.30</td>
<td>57.30±1.37</td>
</tr>
<tr>
<td></td>
<td>18 hr</td>
<td>58.99±1.48</td>
<td>60.17±1.20</td>
</tr>
<tr>
<td>32&lt;sup&gt;nd&lt;/sup&gt; day</td>
<td></td>
<td>60.00±2.15</td>
<td>62.91±1.40</td>
</tr>
</tbody>
</table>

Mean differed non-significantly (p> 0.05) between the groups.
Fig. 4.6: Mean (±SE) serum glucose concentration (mg/dl) of different groups on day of embryo transfer (day 7) at different intervals in crossbred embryo recipient.

Fig. 4.7: Mean (±SE) serum glucose concentration (mg/dl) of different groups on 16th, 17th, 18th and 32nd day of estrus in crossbred embryo recipient.
3.3 Total Serum Cholesterol

The mean serum cholesterol concentration in Control, T-I and T-II group before embryo transfer at zero hour and half hour after treatment was 142.26±3.72, 157.38±6.67 and 150.76±5.72 and 144.67±5.18, 160.79±3.33 and151.60±9.43 mg/dl respectively. There was no significant difference between the groups.

The mean serum cholesterol concentration at 15 minutes, 50 minutes and 150 minutes after transfer in Control, T-I and T-II group was 163.31±2.92, 162.47±2.96 and 154.92±5.17 and 166.98±1.82, 171.44±13.07 and 153.09±5.52 and 169.87±1.74, 161.10±4.96 and 155.80±6.14 mg/dl respectively and there was no significant difference between the groups (Fig 4.8).

The mean cholesterol concentration on 16th, 17th and 18th day of estrus at 0 (zero) hour, 6 hour, 12 hour and 18 hour in Control, T-I and T-II was non-significant difference between the group. On 32nd day the mean cholesterol concentration in Control, T-I and T-II was 156.48±4.29, 159.37±3.15 and 157.95±6.26 mg/dl respectively which differ non-significant between the groups. The mean serum cholesterol concentration remained same between treated and control groups. The highest mean concentration of cholesterol was recorded in T-I (171.44±13.07 mg/dl) than Control (169.87±1.74 mg/dl) and then T-II (157.95±6.26 mg/dl) (Table 4.7 and Fig 4.9)

The mean serum cholesterol concentration recorded in above study were in agreement with reports of Kappel et al. (1984), Kumar (2002), Alameen and Abdelatif (2012) and Sheetal (2017). However, lower concentration has been reported by Pandey et al., (2016) in pregnant and non pregnant crossbred dairy cows and Abd Ellah et al. (2010) in buffalos during different stages of estrous cycle. The dietary fat is also responsible for increase in serum cholesterol level. Leroy et al. (2010) observed that the feeding of FatCH and Fat nearly doubles the cholesterol concentration as compared to control bovines (167.1±11.9; 150±12.8; 83.4±13.7 mg/dl respectively P, 0.05).

There was significant reduction in total cholesterol, total glyceride and low-density lipoproteins (LDL) concentrations in the plasma of experimentally induced hyperlipidemic rats after administrating NASAIDs intraperitoneally (Kourounakis et al., 2002).
Table 4.7: Mean (±SE) serum cholesterol concentration (mg/dl) of different groups on day of transfer (day 7), 16\textsuperscript{th}, 17\textsuperscript{th}, 18\textsuperscript{th} and 32\textsuperscript{nd} day post estrus in crossbred recipients.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>Control</th>
<th>T-I</th>
<th>T-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before transfer</td>
<td>Before treatment</td>
<td>0 hr</td>
<td>142.26±3.72</td>
<td>157.38±6.67</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>1/2 hr</td>
<td>144.67±5.18</td>
<td>160.79±3.33</td>
</tr>
<tr>
<td>After transfer</td>
<td>Day of transfer</td>
<td>15 min</td>
<td>163.31±2.92</td>
<td>162.47±2.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 min</td>
<td>166.98±1.82</td>
<td>171.44±13.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150 min</td>
<td>169.87±1.74</td>
<td>161.10±4.96</td>
</tr>
<tr>
<td>15\textsuperscript{th} day</td>
<td>0 hr</td>
<td>149.50±8.02</td>
<td>158.11±4.62</td>
<td>150.97±7.58</td>
</tr>
<tr>
<td></td>
<td>6 hr</td>
<td>153.70±8.52</td>
<td>153.81±3.65</td>
<td>154.59±4.78</td>
</tr>
<tr>
<td></td>
<td>12 hr</td>
<td>153.60±8.02</td>
<td>161.10±1.32</td>
<td>153.65±7.59</td>
</tr>
<tr>
<td></td>
<td>18 hr</td>
<td>153.23±6.65</td>
<td>159.11±3.92</td>
<td>152.86±3.02</td>
</tr>
<tr>
<td>16\textsuperscript{th} day</td>
<td>0 hr</td>
<td>149.82±6.12</td>
<td>159.29±4.55</td>
<td>146.88±5.94</td>
</tr>
<tr>
<td></td>
<td>6 hr</td>
<td>153.44±4.00</td>
<td>161.00±6.09</td>
<td>151.31±3.53</td>
</tr>
<tr>
<td></td>
<td>12 hr</td>
<td>154.02±4.29</td>
<td>155.01±6.96</td>
<td>150.29±7.78</td>
</tr>
<tr>
<td></td>
<td>18 hr</td>
<td>153.91±4.20</td>
<td>161.13±4.09</td>
<td>148.92±6.81</td>
</tr>
<tr>
<td>17\textsuperscript{th} day</td>
<td>0 hr</td>
<td>152.86±5.21</td>
<td>162.26±5.83</td>
<td>148.77±5.41</td>
</tr>
<tr>
<td></td>
<td>6 hr</td>
<td>154.80±5.43</td>
<td>158.74±5.73</td>
<td>149.29±3.10</td>
</tr>
<tr>
<td></td>
<td>12 hr</td>
<td>152.39±5.78</td>
<td>165.35±6.17</td>
<td>155.07±6.61</td>
</tr>
<tr>
<td></td>
<td>18 hr</td>
<td>160.21±2.64</td>
<td>160.21±3.96</td>
<td>146.93±7.32</td>
</tr>
<tr>
<td>32\textsuperscript{nd} day</td>
<td></td>
<td>156.48±4.29</td>
<td>159.37±3.15</td>
<td>157.95±6.26</td>
</tr>
</tbody>
</table>

Mean differed non-significantly (p> 0.05) between the groups.
Fig. 4.8: Mean (±SE) serum cholesterol concentration (mg/dl) of different groups on day of embryo transfer (day 7) at different intervals in crossbred embryo recipient.

Fig. 4.9: Mean (±SE) serum cholesterol concentration (mg/dl) of different groups on 16th, 17th, 18th and 32nd day of estrus in crossbred embryo recipient.
There was no significant change observed in control and treated group after injecting flunixin meglumine, it may be due to that the drug was given in therapeutic concentration and repetition was also less. Wojnar et al. (1980) reported that higher concentrations of NSAIDs are capable of inducing histamine release from the mast cells, which could potentially aggravate inflammatory response instead of inhibiting it and thus possibly also increasing lipidemia in the animals. Anti-inflammatory drugs such as tolfenamic acid and nimesulide posses antioxidant properties (Rekka et al., 1995), while other drugs like indomethacin, ibuprofen, and diclofenac do not possess such activity (Kourounakis et al., 1999), a fact that may exclude antioxidant activities as the primary mechanism of their hypolipidemic action. NSAIDs have previously been shown to potentially activate the peroxisome proliferator-activated receptor i.e. α and γ (PPARs) (Lehmann et al., 1997) and act as ligand activated transcription factors that mainly modulate the transcription of genes involved in fatty acid metabolism (Lefevre et al., 1997; Vidal-Puig et al., 1997).

3.4 Total Serum Creatinine

The mean serum creatinine concentration in Control, T-I and T-II group before transfer at zero hour and half hour after treatment was 1.53±0.05, 1.62±0.06 and 1.60±0.02 and 1.57±0.03, 1.89±0.34 and 1.61±0.02 mg/dl respectively. There was no significant difference between the groups.

The mean serum creatinine concentration at 15 minutes, 50 minutes and 150 minutes after transfer in Control, T-I and T-II group was 1.56±0.03, 1.61±0.02 and 1.64±0.02 and 1.62±0.02, 1.64±0.03 and 1.69±0.03 and 1.63±0.03, 1.65±0.04 and 1.64±0.04 mg/dl respectively and there was no significant difference between the groups (Fig 4.10).

The mean creatinine concentration on 16th, 17th and 18th day of estrus at 0 (zero) hour, 6 hour, 12 hour and 18 hour in Control, T-I and T-II was non-significant difference between the group. On 32nd day the mean creatinine concentration in Control, T-I and T-II was 1.58±0.03, 1.61±0.05 and 1.60±0.02 mg/dl respectively and there was no significant difference between the groups. The mean serum creatinine concentration remained same between treated and control groups (Table 4.8 and Fig 4.11).
Table 4.8: Mean (±SE) serum creatinine concentration (mg/dl) of different groups on day of transfer (day 7), 16th, 17th, 18th and 32nd day post estrus in crossbred recipients.

<table>
<thead>
<tr>
<th></th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td><strong>Before transfer</strong></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>0 hr</td>
</tr>
<tr>
<td></td>
<td>1/2 hr</td>
</tr>
<tr>
<td>After treatment</td>
<td></td>
</tr>
<tr>
<td>Day of transfer</td>
<td>15 min</td>
</tr>
<tr>
<td></td>
<td>50 min</td>
</tr>
<tr>
<td></td>
<td>150 min</td>
</tr>
<tr>
<td><strong>16th day</strong></td>
<td>0 hr</td>
</tr>
<tr>
<td></td>
<td>6 hr</td>
</tr>
<tr>
<td></td>
<td>12 hr</td>
</tr>
<tr>
<td></td>
<td>18 hr</td>
</tr>
<tr>
<td><strong>17th day</strong></td>
<td>0 hr</td>
</tr>
<tr>
<td></td>
<td>6 hr</td>
</tr>
<tr>
<td></td>
<td>12 hr</td>
</tr>
<tr>
<td></td>
<td>18 hr</td>
</tr>
<tr>
<td><strong>18th day</strong></td>
<td>0 hr</td>
</tr>
<tr>
<td></td>
<td>6 hr</td>
</tr>
<tr>
<td></td>
<td>12 hr</td>
</tr>
<tr>
<td></td>
<td>18 hr</td>
</tr>
<tr>
<td><strong>32nd day</strong></td>
<td></td>
</tr>
</tbody>
</table>

Mean differed non-significantly (p> 0.05) between the groups.
Fig. 4.10: Mean (±SE) serum creatinine concentration (mg/dl) of different groups on day of embryo transfer (day 7) at different intervals in crossbred embryo recipient.

Fig. 4.11: Mean (±SE) serum creatinine concentration (mg/dl) of different groups on 16th, 17th, 18th and 32nd day of estrus in crossbred embryo recipient.
There was a slight increase in the mean creatinine level in T-I and T-II group as compared to control after administration of flunixin meglumine on the day of embryo transfer. In T-II there is slight increase in creatinine concentration on day 16th, 17th and 18th at different hours after injecting flunixin meglumine as compared to T-I and Control. The highest mean concentration of creatinine was recorded in T-I (1.89±0.34 mg/dl) than T-II (1.70±0.02 mg/dl) and then Control (1.63±0.03 mg/dl).

The mean serum creatinine concentration found in the present study was in agreement with the value reported by Sreedhar et al. (2013) in cows during adaptability and in Nellor cows related to age factor (Saraiva et al., 2014) but higher level has reported by Singh et al. (2013) in Hariana Cattle Heifers. Increased creatinine level has been observed in goats (Safarchi et al., 2010), in dogs (Erdogan et al., 2003), in rhabdomyolysis rat model (Tajik et al., 2013) but decreased level has been observed in foals (Carrick et al., 1989) after using flunixin meglumine.

The analysis of mean serum creatinine values does not show any statistical difference (P>0.05) between control and treated groups in rats (Torres et al., 2013). Abo-El-Sooud and Al-Anati, (2011) reported that there was no significant change in serum creatinine in calves after using flunixin meglumine. However, in high producer dairy cows lower values of creatinine were observed (Nozad et al., 2012) and in buffaloes during different stages of estrous cycle (Abd Ellah et al., 2010). The higher activity of creatine kinase in dairy cows was associated with lower production of transferable embryos (Chorfi et al., 2007).

4.5 Total Serum Urea

The mean serum urea concentration in Control, T-I and T-II group before transfer at zero hour and half hour after treatment was 18.44±0.42, 20.34±0.78 and 19.86±0.76 and 18.38±0.46, 21.22±0.72 and 20.25±0.96 mg/dl respectively. There was no significant difference between the groups.

The mean serum urea concentration at 15 minutes, 50 minutes and 150 minutes after transfer in Control, T-I and T-II group was 18.95±0.29, 21.46±0.73 and 20.35±0.90 and 18.93±0.36, 22.08±0.60 and 20.43±1.47 and 18.99±0.71, 22.33±0.86 and 21.53±0.74 mg/dl respectively and there was no significant difference between the groups (Fig 4.12).
Table 4.9: Mean (±SE) serum urea concentration (mg/dl) of different groups on day of transfer (day 7), 16\textsuperscript{th}, 17\textsuperscript{th}, 18\textsuperscript{th} and 32\textsuperscript{nd} day post estrus in crossbred recipients.

<table>
<thead>
<tr>
<th></th>
<th>Urea (mg/dl)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>T-I</td>
<td>T-II</td>
</tr>
<tr>
<td>Before transfer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment 0 hr</td>
<td>18.44±0.42</td>
<td>20.34±0.78</td>
<td>19.86±0.76</td>
<td></td>
</tr>
<tr>
<td>After treatment 1/2 hr</td>
<td>18.38±0.46</td>
<td>21.22±0.72</td>
<td>20.25±0.96</td>
<td></td>
</tr>
<tr>
<td>After transfer Day of transfer 15 min</td>
<td>18.95±0.29</td>
<td>21.46±0.73</td>
<td>20.35±0.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16\textsuperscript{th} day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 hr</td>
<td>18.73±0.94</td>
<td>21.00±0.81</td>
<td>20.04±0.81</td>
<td></td>
</tr>
<tr>
<td>6 hr</td>
<td>19.27±0.99</td>
<td>21.08±1.25</td>
<td>21.72±0.84</td>
<td></td>
</tr>
<tr>
<td>12 hr</td>
<td>19.41±1.29</td>
<td>21.25±0.71</td>
<td>21.99±0.99</td>
<td></td>
</tr>
<tr>
<td>18 hr</td>
<td>18.61±0.56\textsuperscript{b}</td>
<td>20.24±0.61\textsuperscript{b}</td>
<td>22.22±0.87\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>17\textsuperscript{th} day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 hr</td>
<td>19.41±0.45\textsuperscript{b}</td>
<td>20.16±0.67\textsuperscript{b}</td>
<td>22.68±0.84\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>6 hr</td>
<td>19.70±0.46\textsuperscript{b}</td>
<td>21.26±1.01\textsuperscript{b}</td>
<td>23.46±0.88\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>12 hr</td>
<td>18.86±0.75\textsuperscript{b}</td>
<td>19.64±0.83\textsuperscript{b}</td>
<td>23.27±0.79\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>18 hr</td>
<td>19.31±0.91\textsuperscript{b}</td>
<td>19.99±0.80\textsuperscript{b}</td>
<td>23.70±0.81\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>18\textsuperscript{th} day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 hr</td>
<td>20.70±0.71\textsuperscript{b}</td>
<td>18.92±0.75\textsuperscript{b}</td>
<td>23.14±0.40\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>6 hr</td>
<td>21.90±1.14\textsuperscript{b}</td>
<td>20.38±0.65\textsuperscript{b}</td>
<td>23.63±0.40\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>12 hr</td>
<td>20.38±0.63</td>
<td>22.39±0.91</td>
<td>23.42±0.40</td>
<td></td>
</tr>
<tr>
<td>18 hr</td>
<td>19.98±0.62</td>
<td>21.93±1.08</td>
<td>23.45±0.52</td>
<td></td>
</tr>
<tr>
<td>32\textsuperscript{nd} day</td>
<td>19.80±0.83</td>
<td>20.72±0.49</td>
<td>20.88±0.92</td>
<td></td>
</tr>
</tbody>
</table>

Mean differed non-significantly (p> 0.05) between the groups.
**Fig. 4.12:** Mean (±SE) serum urea concentration (mg/dl) of different groups on day of embryo transfer (day 7) at different intervals in crossbred embryo recipient.

**Fig. 4.13:** Mean (±SE) serum urea concentration (mg/dl) of different groups on 16th, 17th, 18th and 32nd day of estrus in crossbred embryo recipient.
The mean urea concentration on 16th, 17th and 18th day of estrus at 0 (zero) hour, 6 hour, 12 hour and 18 hour in Control, T-I and T-II was non-significant difference between the group. On 32nd day the mean urea concentration in Control, T-I and T-II was 19.80±0.83, 20.72±0.49 and 20.88±0.92 mg/dl respectively and there was no significant difference between the groups. The mean serum urea concentration remained same between treated and control groups. There was a significant difference in mean urea concentration in T-II from day 16th (18 hour) to day 18th (6 hour) as compared to T-I and Control, T-I and Control differ non-significantly during this interval (Table 4.9 and Fig 4.13).

There was a slight increase in the mean urea level in T-I and T-II group as compared to control after administration of flunixin meglumine on the day of embryo transfer. In T-II there was a slight increase in urea concentration on day 16th, 17th and 18th at different hours after injecting flunixin meglumine as compared to T-I and Control. The highest mean concentration of urea was recorded in T-II (23.70±0.81 mg/dl) than T-I (22.39±0.91 mg/dl) and then Control (21.90±1.14 mg/dl).

The mean serum urea concentration observed in the present study were in agreement with values reported by Shreedhar et al. (2013) in cattle but lower than reported by Singh et al. (2013) in Hariana Cattle Heifers. There was a significant difference in the blood urea nitrogen in goats treated with flunixin meglumine (Safarchi et al., 2010) and in dogs (Erdogan et al., 2003). The mean urea values showed statistically significant differences between the control and flunixin treated groups in wistar rats (Torres et al., 2013), there was no effect in serum urea concentration using single injection of flunixin and carprofen in dairy cattle (Giammarco et al., 2016).

Long-term exposure of the anti-inflammatory drug induces kidney tumours in rats and liver tumours in mice (Kari et al., 1995). Renal crest necrosis has been reported as a indication of nephropathy caused by NSAIDs in humans (Clive and Stoff, 1984; Carmichael and Shankel, 1985) and in horses (Gunson, 1983; Read, 1983). Renal disease causes impaired blood supply, mainly in the medulla of the kidney (Clive and Stoff, 1984). Renal prostaglandins do not exert any control over basal renal blood flow and glomerular filtration rate in healthy animals and human beings (Gunson, 1983; Clive and Stoff, 1984). In response to renal hypoperfusion, however
there is a increase in local production of prostaglandins in kidney, which act as autoregulators to increase renal perfusion. Inhibition of prostaglandin synthesis by NSAIDs results in decreased ability of the kidneys to autoregulate the blood flow (Gunson, 1983; Clive and Stoff, 1984). Administration of flunixin at low dose does not significantly affect liver and kidney (Erdogan et al., 2003).

Increased in urea concentration was observed after injecting flunixin meglumine but it was within the range, so that embryos would not be affected. The mean serum urea concentration was higher in treated as compared to control cows which was similar to reported by Abo-El-Sooud and Al-Anati, (2011) in calves. The higher urea concentration may lower the chance of pregnancy. In sever negative energy balance cows, the blood urea concentration become higher (Fenwick et al., 2008). The higher blood urea nitrogen level is positively correlated with decrease in fertility in cows (Sawa et al., 2011). Armstrong et al. (2001) and Sinclair et al. (2000) reported that urea has been toxic for oocytes and also embryos recovered from superovulated cows. The cows can recuperate depending on the period of dietary urea exposure (Dawuda et al., 2002). The higher blood urea level significantly reduces the pregnancy rate in cattle (Butler et al., 1996).

4. Hormonal profile

PGFM and progesterone were analysed before and after flunixin meglumine treatment.

4.1 Prostaglandin fetal metabolite (PGFM)

PGFM

The mean serum PGFM concentration in Control, T-I and T-II group before transfer at zero hour and half hour after treatment was 454.63±90.09, 304.36±89.46 and 294.88±59.32 and 441.62±103.38, 376.44±102.78 and 324.49±94.84 pg/ml respectively. There was no significant difference between the groups.

The mean serum PGFM concentration at 15 minutes, 50 minutes and 150 minutes after transfer in Control, T-I and T-II group was 488.41±113.83, 402.09±120.56 and 329.11±108.62 and 457.35±134.86, 477.54±142.28 and 327.88±93.94 and 479.42±147.11,
382.38±89.18 and 364.94±112.64 pg/ml respectively and there was no significant difference between the groups. At 15 minutes there was increase in PGFM in all the groups, at 50 minutes there was decrease in level of PGFM and then at 150 minutes there was again rise in PGFM concentration it is due to uterine manipulation during embryo transfer, but in T-I and T-II the rise was less as compared Control (Fig 4.14).

The mean PGFM concentration on 16^{th}, 17^{th} and 18^{th} day of estrus at 0 (zero) hour and 12^{th} hour in Control, T-I and T-II there was no significant difference between the group. In group T-II there was mark decrease in PGFM concentration after flunixin meglumine injection i.e. on 16^{th} day at 0 hour it was 317.67±59.68 and at 12^{th} hour 213.02±48.86. On 17^{th} day at 0 hour and 12^{th} hour it was 307.61±61.06 and 243.07±41.20 respectively and on 18^{th} day it was 367.01±108.15 and 289.66±54.22 at 0 hour and 12^{th} hour respectively. This decrease in PGFM concentration was not observed in Control and T-I group on 16^{th}, 17^{th}, and 18^{th} day. The highest mean concentration of PGFM was recorded in Control (488.41±113.83 pg/ml) followed by T-I (402.09±120.56 pg/ml) and T-II (367.01±108.15 pg/ml) Table 4.10 and fig 4.15).

The mean serum PGFM concentration recorded in the present study was in agreement with Smith et al. (1979) and Mason et al. (2014) but higher than Parkinson et al. (1990). Mean PGFM concentrations decreased after giving aspirin orally and remained low for 11 h after last aspirin dose, whereas in the control PGFM remained unchanged on day 14 to 15 after standing estrus, it may prevent early luteal tissue regression (Spencer et al., 2016). The variation exists between the individual cows in the number and magnitude of PGFM peaks (Smith et al., 1979), nonpregnant animals have more peaks and variations in the PGFM release (Ellinwood et al., 1979). Prostaglandin is released in pulses from uterine endometrium and 80% of it is metabolized at the time of single passage of the lungs, which helps in creating a short half-life for PGFM as well as fluctuations in its concentration (Nett et al., 1976). There are four factors that affect the release of PGF2α from the uterus, (a) Ovarian steroid hormones, (b) Oxytocin, (c) Mechanical stimulation and (d) Bacterial endotoxin (Roberts et al., 1975).
Table 4.10: Mean (±SE) serum PGFM concentration (pg/ml) of different groups on day of transfer (day 7), 16th, 17th and 18th day of estrus in crossbred recipients.

<table>
<thead>
<tr>
<th></th>
<th>PGFM (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Before transfer</td>
<td>Before treatment 0 hr</td>
</tr>
<tr>
<td></td>
<td>After treatment 1/2 hr</td>
</tr>
<tr>
<td>After transfer</td>
<td>Day of transfer 15 min</td>
</tr>
<tr>
<td></td>
<td>50 min</td>
</tr>
<tr>
<td></td>
<td>150 min</td>
</tr>
<tr>
<td>16th day</td>
<td>0 hr</td>
</tr>
<tr>
<td></td>
<td>12 hr</td>
</tr>
<tr>
<td>17th day</td>
<td>0 hr</td>
</tr>
<tr>
<td></td>
<td>12 hr</td>
</tr>
<tr>
<td>18th day</td>
<td>0 hr</td>
</tr>
<tr>
<td></td>
<td>12 hr</td>
</tr>
</tbody>
</table>

Mean differed non-significantly (p> 0.05) between the groups.

Fig. 4.14: Mean (±SE) serum PGFM concentration (pg/ml) of different groups on day of transfer (day 7) at different intervals in crossbred embryo recipient.
The two injections of flunixin meglumine on 16th and 17th day had effectively decreased the secretion of PGF2α from the uterus or within the ovaries. The oxytocin-induced increase of PGFM concentrations in cows on day 5 after insemination was decreased by injecting flunixin meglumine (Lemaster et al., 1999). Oxytocin secreted by corpus luteum and/or by pituitary gland is potential activator of prostaglandin synthesis and it can be apparently blocked by giving flunixin meglumine. Likely, excessive feeding of flunixin meglumine granules to dairy heifers for nine days, starting on 15th day of the estrus cycle have delayed luteolysis, decreased the number of PGFM pulses and significantly increased the length of the cycle (Odensvik et al., 1998). Mann and Lamming (2001) reported that some embryos were so poorly developed that the concentration of IFN-τ was either undetectable or very low in uterine flushings on 16th day after insemination. The cows with measurable IFN-τ concentration had an altered PGFM response to Oxytocin whereas cows with very low or undetectable IFN-τ concentration were similar as normal cyclic cows. It was concluded that MRP associated with the maintenance of a good quality corpus luteum is only successful when embryo is well developed and is able to secrete appropriate amounts of IFN-τ. Poorly developed embryo may be
viable and develop slowly, so due to this maternal recognition of pregnancy induced by IFN-τ does not start at appropriate time.

In the present study, the injection of flunixin meglumine may have exerted an inhibitory effect on PGFM and decreased its level. The excessive use of the drug delays luteolysis and increases the length of estrous cycle in dairy heifers (Aiumlamai et al., 1990; Odensvik et al., 1998). Feeding flunixin meglumine orally decreased the concentration of PGFM which remained low for 10 to 30 hours in dairy heifers (Odensvik, 1995), this indicates that injecting flunixin meglumine 24 hours apart have resulted in lower concentrations of PGFM. At the time of luteolysis, PGF2α secretion continues for about two to three days (Basu and Kindahl, 1987).

4.2 Progesterone

Progesterone concentration directly reflects the function of corpus luteum and is precise indicator of ovarian function. In present study mean serum progesterone concentration in Control, T-I and T-II before transfer at zero hour and 150 minutes after transfer/treatment was 1.17±0.68, 1.17±0.31 and 1.65±0.53 and 1.98±1.40, 1.98±0.58 and 1.65±0.41 ng/ml respectively and differ non significantly.

The mean serum progesterone concentration on treatment day at 16th and 17th day of estrus in Control, T-I and T-II was 2.21±0.76, 1.76±0.62 and 1.88±0.45 and 3.67±0.90, 2.54±0.62 and 2.75±0.97 ng/ml respectively and there was no significant difference between the groups but increase in progesterone concentration was observed in T-II group.

On 18th day mean serum progesterone level in Control and T-I was 2.70±1.14, 2.53±0.78 respectively in T-II group there was marked increases in progesterone level was recorded i.e. 3.62±1.26. On day 32nd the mean serum concentration progesterone level higher in T-II group i.e. 4.13±2.23 as compared to Control and T-I 2.69±1.82 and 1.15±0.70 respectively (Table 4.11 and Fig 4.16).

One cow has been excluded from control group because the serum progesterone was 1.773, 3.083, 10.37, 18.48, 9.843 and 4.089 on day of transfer before treatment, 150 minute after transfer/treatment, 16th, 17th, 18th and 32nd day post transfer indicative of cystic ovarian degeneration. Five cows were taken in control group for statistical analysis.
Table 4.11: Mean (±SE) serum progesterone concentration (ng/ml) of different groups on day of transfer (day 7), 16th, 17th, 18th and 32nd day of estrus in crossbred recipients.

<table>
<thead>
<tr>
<th></th>
<th>Progesterone (ng/ml)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>T-I</td>
<td>T-II</td>
<td></td>
</tr>
<tr>
<td>Before transfer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td></td>
<td>0 hr</td>
<td>1.17±0.68</td>
<td>1.17±0.31</td>
</tr>
<tr>
<td>After transfer</td>
<td></td>
<td>150 min</td>
<td>1.98±1.40</td>
<td>1.98±0.58</td>
</tr>
<tr>
<td>16th day</td>
<td></td>
<td>12 hour</td>
<td>2.21±0.76</td>
<td>1.76±0.62</td>
</tr>
<tr>
<td>17th day</td>
<td></td>
<td>12 hour</td>
<td>3.67±0.90</td>
<td>2.54±0.62</td>
</tr>
<tr>
<td>18th day</td>
<td></td>
<td>12 hour</td>
<td>2.70±1.14</td>
<td>2.53±0.78</td>
</tr>
<tr>
<td>32nd day</td>
<td></td>
<td>2.69±1.82</td>
<td>1.15±0.70</td>
<td>4.13±2.23</td>
</tr>
</tbody>
</table>

Mean differed non-significantly (p > 0.05) between the groups.

Control n=5; T-I n=6; T-II n=3.

Fig. 4.16: Mean (±SE) serum progesterone concentration (ng/ml) of different groups on 16th, 17th, 18th and 32nd day of estrus in crossbred embryo recipient.
Rajamahendra et al. (1976) have reported progesterone level less than 1 ng/ml during first two days of the cycle, it increases rapidly over 4th to 12th day and reaches a peak level of 5.2±1.1 ng/ml on day 14 of the cycle. Thereafter, the level declined to 2.6±0.6 ng/ml on day 16 and to 0.40±0.1 ng/ml on day 21 of the cycle. Agarwal et al. (1982) have also reported the value 0.82, 4.67 and 4.59 ng/ml on day 1, 13 and 16 of the estrous cycle, in Jersey X Sahiwal crossbred cows. The plasma progesterone concentration in 47 Bos Indicus x Bos Taurus embryo recipient cattle did not vary on day 0 (estrus), day 7 of estrous cycle in pregnant and non-pregnant cattle whereas the progesterone concentration on day 21 was 12.2 ng/ml in pregnant females vs 0.82 ng/ml for non-pregnant females. Plasma progesterone concentrations did not differ between non-pregnant (1.31±0.9) and pregnant (1.50±1.05ng/mL) Nellore and crossbreed cattle (Nogueira et al., 2012). O’Hara et al. (2014) reported that injecting hCG on Day 2 of estrus approximately doubled conceptus length on Day 14 (4.94±1.15 mm; P < 0.05), whereas inserting PRID from Days 3 to 5 of estrus increases conceptus length about fivefold (13.09±2.11 mm; P < 0.05) compared with control. It indicates that supplemental progesterone is capable of “rescuing” poor-quality blastocysts.

5. Conception following transfer of crossbred embryos.

Eighteen crossbred embryos were transferred to eighteen healthy crossbred cows. Cows were divided into three groups i.e. control (n=6), T-I (n=6) and T-II (n=6). In control no conception was found, in T-I one cow conceived but aborted after 76 days and in T-II two pregnancies were confirmed.

5.1 Expected gain from the progenies

The progenies that will be born through embryo transfer in present study would be elite, as genetic material of embryo donor cow and semen that was used for breeding was of good production potential. Recorded milk production of donor animal was 3044.01 lit and 3211.17 lit/ lactation (305 days) respectively and the Dam’s yield of Sire was 13849.00 lit/ lactation (305 days). So the expected production potential of progeny would be 8446.50 lit and 8530.08 lit/ lactation (305 days) respectively.

Thus, the elite progenies produce would be utilized for genetic improvement of livestock in the vicinity of the University. Also, nuclear herd of elite animals may be maintained at I.D.F. Nagla for wider dissemination of germ plasm (Annexure 1).

Results and Discussion …………….
6. The effect of flunixin meglumine on PGFM and progesterone level in Control non-pregnant, Treated non-pregnant and Treated pregnant/expected pregnancy

The average serum PGFM concentration in Control non-pregnant, Treated non-pregnant (T-II) and Treated pregnant/expected pregnancy (T-II) before transfer at zero hour and half hour after treatment was 419.63±90.09, 346.22±108.33 and 243.53±16.95 and 441.62±103.38, 395.98±191.62 and 280.08±57.72 pg/ml respectively.

The average serum PGFM concentration at 15 minutes, 50 minutes and 150 minutes after transfer in Control, T-I and T-II group was 488.41±113.83, 395.98±191.62 and 262.23±89.23 and 457.35±134.86, 384.61±175.11 and 271.15±53.71 and 479.42±147.11, 349.51±120.87 and 292.36±77.89 pg/ml respectively. At 15 minutes there was increase in PGFM in all the groups, at 50 minutes there was decrease in level of PGFM but a very slight increase was observed in Treated pregnant/expected pregnancy (T-II) group and then at 150 minutes there was again rise in PGFM concentration which might be due to uterine manipulation during embryo transfer.

The average PGFM concentration on 16th, 17th and 18th day of estrus at 0 (zero) hour and 12th hour in Control non-pregnant, Treated non-pregnant (T-II) and Treated pregnant/expected pregnancy (T-II), there was observable difference in serum level between the group. In Treated non-pregnant (T-II) and Treated pregnant/expected pregnancy (T-II) there was marked decrease in PGFM concentration after flunixin meglumine injection i.e. on 16th day at 0 hour it was 313.03±103.68 and 322.30±63.77 and at 12th hour 206.13±96.26 and 219.90±25.13. On 17th day at 0 hour and 12th hour it was 299.26±106.67 and 315.95±63.93 and 209.88±43.03 and 276.25±64.18 respectively and on 18th day it was 450.66±178.15 and 283.36±100.01 and 284.00±46.21 and 295.32±100.40 at 0 hour and 12th hour respectively (Table 4.12, Fig 4.17).

The average serum progesterone concentration in Control non-pregnant, Treated non-pregnant (T-II) and Treated pregnant/expected pregnancy (T-II) before transfer at zero hour and 150 minutes after transfer/treatment was 1.17±0.68, 1.92±0.85 and 1.33±0.57 and 1.98±1.40, 1.58±0.71 and 1.71±0.44 ng/ml respectively.

The average progesterone concentration on treatment day at 16th and 17th day of estrus in Control non-pregnant, Treated non-pregnant (T-II) and Treated pregnant/expected pregnancy (T-II) was 2.21±0.76, 1.15±0.37 and 2.61±0.41 and 3.67±0.90, 1.25±1.07 and 4.24±0.72 ng/ml respectively.
Table 4.12: Average serum PGFM concentration (pg/ml) in Control non-pregnant, Treated non-pregnant (T-II) and Treated pregnant/expected pregnancy (T-II) before and after transfer in crossbred recipient.

<table>
<thead>
<tr>
<th></th>
<th>PGFM (pg/ml)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control non-pregnant</td>
<td>Treated non-pregnant (T-II)</td>
<td>Treated pregnant/expected pregnancy (T-II)</td>
<td></td>
</tr>
<tr>
<td>Before transfer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>0 hr</td>
<td>419.63±90.09</td>
<td>346.22±108.33</td>
<td>243.53±16.95</td>
</tr>
<tr>
<td></td>
<td>1/2 hr</td>
<td>441.62±103.38</td>
<td>368.89±179.37</td>
<td>280.08±57.72</td>
</tr>
<tr>
<td>After treatment</td>
<td>15 min</td>
<td>488.41±113.83</td>
<td>395.98±191.62</td>
<td>262.23±89.23</td>
</tr>
<tr>
<td></td>
<td>50 min</td>
<td>457.35±134.86</td>
<td>384.61±175.11</td>
<td>271.15±53.71</td>
</tr>
<tr>
<td></td>
<td>150 min</td>
<td>479.42±147.11</td>
<td>349.51±120.87</td>
<td>292.36±77.89</td>
</tr>
<tr>
<td>After Day of transfer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16th day</td>
<td>0 hr</td>
<td>289.44±67.56</td>
<td>313.03±103.68</td>
<td>322.30±63.77</td>
</tr>
<tr>
<td></td>
<td>12 hr</td>
<td>242.32±56.42</td>
<td>206.13±96.26</td>
<td>219.90±25.13</td>
</tr>
<tr>
<td>17th day</td>
<td>0 hr</td>
<td>232.76±38.77</td>
<td>299.26±106.67</td>
<td>315.95±63.93</td>
</tr>
<tr>
<td></td>
<td>12 hr</td>
<td>251.66±66.65</td>
<td>209.88±43.03</td>
<td>276.25±64.18</td>
</tr>
<tr>
<td>18th day</td>
<td>0 hr</td>
<td>336.68±69.59</td>
<td>450.66±178.15</td>
<td>283.36±100.01</td>
</tr>
<tr>
<td></td>
<td>12 hr</td>
<td>219.74±20.47</td>
<td>284.00±46.21</td>
<td>295.32±100.40</td>
</tr>
</tbody>
</table>

Control non-pregnant n=6; Treated non-pregnant (T-II) n=3; Treated pregnant/expected pregnancy (T-II) n=3.

Fig. 4.17: Average serum PGFM concentration (pg/ml) in Control non-pregnant, Treated non-pregnant (T-II) and Treated pregnant/expected pregnancy (T-II) before and after transfer in crossbred recipient.
On 18\textsuperscript{th} day the average progesterone level in Control non-pregnant, Treated non-pregnant (T-II) and Treated pregnant/expected pregnancy (T-II) was 2.70±1.14, 1.98±1.83 and 5.25±0.72 respectively. On day 32\textsuperscript{nd} the average progesterone level was higher in Treated pregnant/expected pregnancy (T-II) group i.e. 8.12±2.19 as compared to Control non-pregnant and Treated non-pregnant (T-II) 2.69±1.82 and 0.13±0.01 respectively (Table 4.13, Fig. 4.18).

In present study it was observed that giving flunixin meglumine at the dose rate of 1.1 mg/kg on 16\textsuperscript{th} and 17\textsuperscript{th} day effectively reduces the PGFM level and simultaneously due to reduction of PGFM level progesterone concentration is increased which would be beneficial for embryo survival and helps in improving conception after embryo transfer. The decrease in PGFM concentration may provide an extra time for week and viable embryos to secrete sufficient amount of IFN-τ to inhibit the luteolytic secretion of PGF2α. It may be possible that flunixin meglumine may only delay luteolysis and increases the survivability of poor quality embryos that would die later during the pregnancy. The conceptus also secretes appreciable amounts of prostaglandins (Lewis \textit{et al.}, 1982). Maternal factors may be responsible to inhibit the luteolytic releases of PGF2α. Mann and Lamming (2001) reported that maternal endocrine environment and the endocrine environment of conceptus, in that more developed conceptuses secrete higher amount of IFN-τ as compared to weak conceptus, which results in higher concentrations of progesterone in the maternal circulation of developed conceptus. On the basis of above findings, it may be concluded that administration of flunixin meglumine during luteal phase at the time of maternal reorganization of pregnancy decreases PGFM level and boost progesterone level which may be beneficial for the conception of embryo following embryo transfer.
Table 4.13: Average serum progesterone concentration (ng/ml) in Control non-pregnant, Treated non-pregnant (T-II) and Treated pregnant/expected pregnancy (T-II) before and after transfer in crossbred recipient.

<table>
<thead>
<tr>
<th></th>
<th>Progesterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control non-pregnant</td>
</tr>
<tr>
<td>Before transfer</td>
<td>0 hr</td>
</tr>
<tr>
<td>After transfer</td>
<td>After treatment/Day of transfer</td>
</tr>
<tr>
<td></td>
<td>150 min</td>
</tr>
<tr>
<td></td>
<td>16th day</td>
</tr>
<tr>
<td></td>
<td>17th day</td>
</tr>
<tr>
<td></td>
<td>18th day</td>
</tr>
<tr>
<td></td>
<td>32nd day</td>
</tr>
</tbody>
</table>

Control non-pregnant n=5; Treated non-pregnant (T-II) n=3; Treated pregnant/expected pregnancy (T-II) n=3.

Fig. 4.18: Average serum progesterone concentration (ng/ml) in Control non-pregnant, Treated non-pregnant (T-II) and Treated pregnant/expected pregnancy (T-II) before and after transfer in crossbred recipient.
Summary and Conclusions
SUMMARY AND CONCLUSION

Improving the genetic potential of crossbred cattle for milk has been a major concern for decades in many countries and has become a recent development focus in several more. Limits to this initiative have been imposed by biological conditions inherited in the animal such as delayed puberty, silent estrus, long postpartum ovarian inactivity and poor fertility. Emerging biotechniques can be an effective way of improving reproductive efficiency and enhancing the production of genetically superior cattle. AI could be one such tool but due to scarcity of elite cattle bull only 20% of cattle population could bred. So, there is an urgent need to produce elite males through adaption of newer reproductive techniques like embryo transfer, ovum pick up, in vitro fertilization etc.

The present study was conducted on crossbred cattle(n=23) maintained at Instructional Dairy Farm (I.D.F.) Nagla, G.B. Pant University of Agriculture and technology, Pantnagar-263145, District- Udham Singh Nagar (Uttarakhand) with the objectives (i) To study the effect of flunixin meglumine on PGF2α, progesterone level following embryo transfer. (ii) To study the alteration in glucose and protein in embryo recipient animals. (iii) To study the effect of flunixin meglumine treatment on conception rate.

EMBRYO DONOR CATTLE

Five (n=5) normal cyclic high pedigreed crossbred cows were selected as donor on the basis of per-rectal examination of their genital organs and their health records. The animals were treated with 240 mg of Folltropin-V/animal, as a total dose and given at 12 hours interval in 8 divided doses in decreasing order i.e. 45:45, 35:35, 25:25, 15:15 mg, morning and evening, by IM route starting from day 9 of estrus cycle (Day 0=day of estrus). The superestrus in donor cows were induced with IM injection of 500 µg cloprostenol with 6th dose of FSH-P.

All animals were superovulated twice, two cycle gap was kept in between second superovulation of each cattle. Animals were subjected to estrus detection and were twice inseminated at 12 hour interval through artificial insemination.
Superovulatory response was examined on sixth day of first A.I. by per-rectal palpation. The embryo collection was done on 7½ day of first artificial insemination. The embryo collection was done as per standard non-surgical procedures and embryos were evaluated.

The overall superovulatory response was 90%. The mean ovulation rate (number of CL) was 9.6 ±1.84, total embryo/ova recovery per animal was 6.3 ±1.84 and the overall embryo recovery rate was 65.62%. A total of 58 transferable embryos were recovered. The mean number of transferable embryos recovered was 5.8±1.81. The average transferable embryos were 60.41%. The mean total 4-16 cell stage, morula, blastocyst, unfertilized, degenerated and empty zona recovered were 0.2±0.20, 2.2±0.57, 3.6±1.36, 0.1±0.10, 0.1±0.10 and 0.1±0.10 respectively. The average recovery of total 4-16 cell stage, morula, blastocyst, unfertilized, degenerated and empty zona were 3.17%, 34.92%, 57.14%, 1.58%, 1.58% and 1.58% respectively.

**EMBRYO RECIPIENT CATTLE**

Eighteen normal cyclic crossbred cows were selected on the basis of their health record and per-rectal examination of genital organs from Instructional Dairy Farm (I.D.F) as embryo recipients. The animals (n=18) were divided into 3 groups as Control (n=6) : NSS was given, T-I (n=6) : flunixin meglumine @ dose rate of 1.1 mg/kg was given half hour before transfer, T-II (n=6) : flunixin meglumine @ dose rate of 1.1 mg/kg was given half hour before transfer and on 16th and 17th day of standing estrus.

Blood sample collection was done; before transfer i.e. on 7½ day of estrus firstly without any treatment then half hour after treatment. After transfer at, 15 minute, 50 minute and 150 minute blood collection was done. On 16th, 17th and 18th day of estrus blood was collected after every 6 hour interval and then on 32th day of standing estrus for protein, glucose, cholesterol, creatinine and urea. For PGFM blood samples were collected on day 7½ of estrus i.e. before transfer at zero hour and half hour after treatment and at 15 minute, 50 minute and 150 minute after transfer of embryo. Blood samples were also collected on day 16, 17 and 18 of estrous cycle, at 12 hour interval. While for estimation of progesterone two blood samples were collected on day of transfer; firstly before transfer at zero hour and second at 150 minute after transfer on day 7½ of estrus. While blood samples were also collected once in a day on 16th, 17th, 18th and 32nd day of estrus cycle.
The mean serum protein concentration in Control, T-I and T-II group before transfer at zero hour and half hour after treatment differ non-significantly (p>0.05) between the groups. The concentration of total protein at 15 minutes, 50 minutes and 150 minutes after transfer in Control, T-I and T-II group were non-significant (p>0.05) but there was slight decrease in T-I and T-II from control. At every 6 hour interval on 16th, 17th and 18th day of estrus there was no significant difference (P>0.05) in the mean serum protein concentration but a increase level of protein concentration was observed in T-II as compared to control and T-I. On day 32 there was no significant (p>0.05) difference between control, T-I and T-II.

The mean concentration of serum glucose differ non-significantly (p>0.05) between control, T-I and T-II group before transfer at zero hour and half hour after treatment and at 15 minutes, 50 minutes and 150 minutes after the transfer. On 16th, 17th and 18th day of estrus at every 6 hour there was no significance (p>0.05) difference found in serum glucose level, similar finding was observed on day 32 of standing estrus.

The level of serum cholesterol differ non-Significantly (p>0.05) on day of transfer before and after treatment and at 15 minutes, 50 minutes and 150 minutes after the transfer between the groups control, T-I, and T-II, and no significance was observed in serum cholesterol values on day 16th, 17th and 18th (p>0.05) at every six hour interval and on day 32 of estrus.

The mean serum creatinine concentration before transfer at zero hour and half hour after treatment did not differ significantly (p>0.05), there was increase in serum creatinine concentration in group T-I and T-II as compared to control after the treatment but no significance (p>0.05) was seen at 15 minutes, 50 minutes and 150 minutes after the transfer between the groups. The higher value of creatinine was recorded in T-II as compared to T-I and control on day 16th and 17th day and becomes normal on 18th day at 6th hour but it does not differ significantly (p>0.05) between the groups and on day 32.

The mean concentration of serum urea increased after treatment in T-I and T-II as compared to control on the day of transfer but there was no significant difference (p>0.05) between the groups. Increase in urea was observed on day 16th, 17th and 18th in group T-II and differ significantly (P<0.05) from control and T-I from 18th hour.
sample of day 16 to 6th hour sample of day 18 hour. And no significance (p>0.05) was observed on day 32 between the groups.

The mean serum PGFM concentration in Control, T-I and T-II group before transfer at zero hour and half hour after treatment differ non-significantly (p>0.05) between the groups. The concentration of total PGFM at 15 minutes, 50 minutes and 150 minutes after transfer in Control, T-I and T-II group were non-significant (p>0.05) but decrease in T-I and T-II from control was recorded. At every 12 hour interval on 16th, 17th and 18th day of estrous there was no significant difference (P>0.05) in the mean serum PGFM concentration but a decreased level of PGFM concentration was observed in T-II after the flunixin injection as compared to control and T-I. On day 32 there was no significant (p>0.05) difference between control, T-I and T-II.

The mean serum progesterone concentration did not differ significantly (p>0.05) in control, T-I and T-II group before treatment/transfer, 150 min after transfer/treatment, 16th, 17th, 18th, and 32nd day of estrous cycle but marked increase was observed in T-II group on 16th, 17th and 18th day of estrus after injecting flunixin meglumine on 16th and 17th day of estrus.

There was no conception in control group, in T-II one animal conceived but aborted after 76 days. In group T-II two animals became pregnant.

On the basis of above findings, it may be concluded that administration of flunixin meglumine during luteal phase at the time of maternal recognition of pregnancy decreases PGFM level and increase progesterone level which may be beneficial for the conception following embryo transfer.

CONCLUSION

1. Good superovulatory response i.e. 9.6±1.84/donor can be achieved by injecting lower total doses of FSH in tapering schedule in crossbred cattle.
2. There is no significant variation in biochemical parameter following flunixin meglumine administration
3. Flunixin meglumine administration during luteal phase of estrous cycle following embryo transfer may be beneficial in improving conception.
Literature Cited


Literature Cited ……………


*Literature Cited ....................*


Salazar-Ortiz, J., Monget, P. and Guillaume, D. 2014. The influence of nutrition on the insulin-like growth factor system and the concentrations of growth hormone, glucose, insulin, gonadotropins and progesterone in ovarian follicular fluid and plasma from adult female horses (Equus caballus). Reproductive biology and endocrinology, 12(1), 72.


Situmorang, P. 2005. Effect the administration of human chronic gonadotrophin (hCG) hormone following superovulation treatment in buffalo. JITV, 10: 286-292.


Literature Cited ……………


Literature Cited ……………


Literature Cited ……………


Annexure
### ANNEXURE 1

**Expected gain from the progenies**

<table>
<thead>
<tr>
<th>Donor name</th>
<th>Donor’s lactation (305 days)</th>
<th>Sire’s (Dam’s yield 305 days)</th>
<th>Recipient name (heifers)</th>
<th>Recipient Dam’s yield (305 days)</th>
<th>Transfer date</th>
<th>Expected delivery</th>
<th>Progeny expected yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand 347 (P.Y= 18.0 lit)</td>
<td>3211.17 lit</td>
<td>13849.00 lit</td>
<td>AP 220</td>
<td>3536.47 lit</td>
<td>2/5/2017</td>
<td>5/2/2018</td>
<td>8530.08 lit</td>
</tr>
<tr>
<td>CBT 589 (P.Y= 19.2 lit)</td>
<td>3044.01 lit</td>
<td>13849.00 lit</td>
<td>AP 665</td>
<td>3051.37 lit</td>
<td>13/5/2017</td>
<td>15/2/2018</td>
<td>8446.50 lit</td>
</tr>
</tbody>
</table>
PEDIGREE DETAIL OF BULLS MAINTAINED AT DFSPC

**Bull: HF-1335**
DoB: 04.01.2014  
Dam No.: 0093/ULDB  
Sire's name / No.: Discover  
Dam's Best Standard Lactation (Kgs.): 6419  
Sire's Dam's Best Standard Lactation (Kgs.):

**Bull: HF-AJAY (ET Produced- Imported)**
DoB: 30.05.2011  
Dam No.: McIntosh Jennilee  
Sire's name / No.: Kerndtway Howie  
Dam's Best Standard Lactation (Kgs.): 13849  
Sire's Daughter Lactation Average (Kgs.): 11635
VITA

The author of this manuscript, Dr. Ankit Malik S/o Mr. Anil Kumar Malik and mother Mrs. Anita Malik, was born on 16th November, 1991 at Haridwar dist. of Uttarakhand. He passed his High School and Intermediate examination from Seventh Day Adventist Inter College, Roorkee in year 2007 and 2009, respectively both with I division. Thereafter, he joined B.V.Sc & A.H. degree programme in 2010, participated in Republic Day Camp in 2010 (RDC) from Remount & Veterinary Corps (RVC) and passed with I division from College of Veterinary Sciences and Animal Husbandry, G.B. Pant University of Agriculture & Technology, Pantnagar-263 145 (U.S. Nagar), Uttarakhand, India. He was awarded Teaching Assistant Fellowship from G.B.P.U & T during his master’s degree. Presently he is member of Uttarakhand Veterinary Council, Indian Society for Study of Animal Reproduction and Veterinary Council of India.

Mailing Address:

Dr. Ankit Malik  
S/o Mr. Anil Kumar Malik  
H.No. -117,Gali No. - 3  
New Mohanpura, Laxminagar  
Roorkee,  
Dist. Haridwar  
UTTARAKHAND  
PIN-247667.  
Mobile no.08979890900  
E-Mail: ankitvet5@gmail.com
The present study was designed to study the effect of flunixin meglumine during embryo transfer and at the time of MRP on PGFM, progesterone and biochemical profile in embryo recipient cows. Crossbred donor cow (n=5) treated with 240 mg of Folltropin-V/animal, as a total dose and given at 12 hours interval in 8 divided doses in decreasing order i.e. 45:45, 35:35, 25:25, 15:15 mg, morning and evening, by IM route, each cow was superovulated twice. Further, crossbred cows (n=18) were selected and divided into three groups; control, Control (n=6) : NSS was given, T-I (n=6) : flunixin meglumine @ dose rate of 1.1 mg/kg was given half hour before transfer, T-II (n=6) : flunixin meglumine @ dose rate of 1.1 mg/kg was given half hour before transfer and on 16th and 17th day of standing estrus. Blood sample collection was done; Before transfer i.e. on 7½ day of estrus without any treatment then half hour after treatment. After transfer, at 15 minute, 50 minute and 150 minute blood collection was done. On 16th, 17th and 18th day of estrus blood was collected after every 6 hour interval and then on 32th day of standing estrus. Blood sample collection was done same as above but on day 16,17 and 18 of estrus cycle blood sampling was done after every 12 hour and for progesterone estimation blood samples were taken before treatment/transfer, 150 min after transfer/treatment then on 16th, 17th, 18th, and 32nd day of estrus.

The overall superovulatory response, mean ovulation, total embryo/ova recovered and average transferable embryos recovered were 90%, 9.6±1.84, 6.3±1.84 and 60.41% respectively. There was no significance difference (p>0.05) in protein, glucose, cholesterol and creatinine serum level but there was slight decrease in protein level while creatinine level increased in treated group. Serum urea concentration differ significantly (p<0.05) between the groups after treatment.

The PGFM level and progesterone level also differ non significantly (p>0.05) between the groups but after treatment there was marked decrease in PGFM level and simultaneously progesterone concentration increased. One animal aborted at 76 days and two became pregnant. On the basis of above findings, it may be concluded that administration of flunixin meglumine during luteal of estrous cycle may be beneficial in improving conception following embryo transfer in crossbred cattle as evident by decreased level of PGFM and increased level of progesterone during critical days of maternal recognition of pregnancy.
सारांश

वर्तमान अवस्था में भूमि आदाता गायों में MRP के समय PGFM, प्रोजेक्टेंटोन तथा अन्य जैव रसायनिक क्रियान्वयन पर भूमि प्रायोगिक क्रम का अवयव किया गया है। संकर दलता गायों (n=5) में कुल 240 निर्माण फील्डटिप्पन-दी प्रत्येक 12 घण्टों के अंतराल में 8 बार चढ़ते हुए क्रम में 44:45, 35:35, 25:25, 15:15 निर्माण सुबह और शाम अंतरालमंदों में दिया गया। प्रत्येक गाय में दो बार गर्भधारण किया गया। इसके अतिरिक्त संकर गायों (n=18) को चयनित कर तीन भागों में विभाजित किया गया जिसके तहत निम्न (n=6) भाग में NSS दिया गया T-I (n=6) भाग में औसत भूमि प्रायोगिक क्रम के आधा घण्टा पूर्व 1.1 निर्माण/किमी की दर से दिया गया जबकि T-II (n=6) भाग में औसत 1.1 निर्माण/किमी की दर से भूमि प्रायोगिक क्रम से आधा घण्टा पूर्व तथा मदकाल में 16वें व 17वें दिन दिया गया। प्रायोगिक क्रम के दिन (7.5) बिना प्रायोगिक क्रम से पहले घण्टे और औसत देने के आधे घण्टे पूर्व एवं भूमि प्रायोगिक क्रम के 15 मिनट, 30 मिनट, 45 मिनट तथा 16वें, 17वें एवं 18वें दिन प्रत्येक 6 घण्टे के अंतराल पर और एक 32वें दिन प्रोटीन, न्यूकोजन, कोलेस्टरल, क्रिटोसीन और यूरिया के आंकन हेतु रक्त के नमूने लिये गये। लेकिन PGFM हेतु प्रायोगिक क्रम के दिन पूर्व की भावना रक्त नमूने लिये गये जबकि मदकाल के 16वें, 17वें एवं 18वें दिन प्रत्येक 12 घण्टे के अंतराल में रक्त के नमूने लिये गये तथा प्रोजेक्टेंटोन के आंकन हेतु भूमि प्रायोगिक क्रम वाले दिन एक रक्त का नमूना 0 घण्टा प्रायोगिक क्रम से पूर्व तथा 150 मिनट औसत/प्रायोगिक क्रम के बाद और एक-एक नमूना 16वें, 17वें एवं 18वें दिन पर लिया गया।

समूहों अंतिम परीक्षा औसत से अधिक तथा औसत भूमि प्रायोगिक क्रम के मान क्रमशः: 90.9, 96 ± 1.84, 63% तथा 60.41% पाये गये। उपचार वाले समूह में प्रोटीन ग्रूपोजन कोलेस्टरल तथा क्रिटोसीन के मान में कोई महत्त्वपूर्ण अंतर (P>0.05) नहीं था जबकि प्रोटीन के मान में मामूली व्यावधि तथा क्रिटोसीन के मान में वृद्धि पायी गयी। उपचारित समूहों में यूरिया में विन्दु (P < 0.05) पायी गयी।

समूहों के बीच PGFM स्तर और प्रोजेक्टेंटोन में भी कोई महत्त्वपूर्ण अंतर नहीं पाया गया (P > 0.05) लेकिन औसत दिन गया समूह में PGFM के स्तर में कमी पायी गयी साथ ही साथ प्रोजेक्टेंटोन की साधन में वृद्धि पायी गयी। एक गाय में 76 दिन बाद गर्भपात हुआ और दो गाये गर्भित मिली। उपचारित आंकन के आधार पर यह निष्कर्ष निकाला जा सकता है कि मदकाल के लूट्सीनवर्त में परुषपूर्ण में मदकाल देने से भूमि प्रायोगिक क्रम से बाद गर्भधारण में फायदा मिल सकता है क्योंकि MRP के महत्त्वपूर्ण दिनों के दौरान PGFM के स्तर में कमी तथा प्रोजेक्टेंटोन के स्तर में वृद्धि देखी गयी जो गर्भधारण के लिये प्रत्यक्ष मूल से उत्तरदायी है।

(शिव शर्मा)
सलाहकार