FERTILITY RESPONSE OF THE SEX SORTED SEMEN IN CROSSBRED CATTLE

T H E S I S

Submitted
In partial fulfillment of the requirements for the Degree of

MASTER OF VETERINARY SCIENCES
IN
ANIMAL REPRODUCTION, GYNAECOLGY AND OBSTETRICS

BY

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Enrollment No. V/19/404

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2021

DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled **"FERTILITY RESPONSE OF THE SEX SORTED SEMEN IN CROSSBRED CATTLE"** or part thereof has not been submitted for any other degree or diploma of any university, nor the data have been derived from any thesis/publication of any University or Scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

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Shri JADHAV SURESH CHANGDEV has satisfactorily prosecuted his course of research for a period of not less than one semester and that the thesis entitled **"FERTILITY RESPONSE OF THE SEX SORTED SEMEN IN CROSSBRED CATTLE"** submitted by him is the result of research work sufficient to warrant its presentation to the examination in the subject of **Animal Reproduction, Gynaecology and Obstetrics** for the award of **Master of Veterinary Science** degree by the Maharashtra Animal and Fishery Sciences University, Nagpur.

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

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CERTIFICATE

This is to certify that the thesis entitled, **"FERTILITY RESPONSE OF THE SEX SORTED SEMEN IN CROSSBRED CATTLE"** submitted by Shri. **JADHAV SURESH CHANGDEV** to the Maharashtra Animal and Fishery Sciences University in partial fulfillment of the requirement for the degree of **MASTER OF VETERINARY SCIENCE** has been approved by the Student's Advisory Committee after examination in collaboration with the External Examiner.

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Place: Udgir

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%	:	Percent
@	:	At the dose rate of
<	:	Less than
>	:	Greater than
±	:	Plus or Minus
≤	:	Less than or equals to
^{0}F	:	Fahrenheit
AI	:	Artificial Insemination
BCS	:	Body Condition Score
BW	:	Body weight
Ca	:	Calcium
CCDC	:	Charge Coupled Device Camera
CIDR	:	Controlled Intravaginal Drug Releasing Device
CON	:	Conventional
CL	:	Corpus luteum
Co	:	Cobalt
CR	:	Conception Rate
Cu	:	Copper
D	:	Day
Dl	:	desiliter
et al.	:	et alia (and others)
Fe	:	Iron
Fig	:	Figure
FTAI	:	Fixed-Time Artificial Insemination
G	:	Gram
GF	:	Graffian follicle
g/dl	:	Gram per deciliter
g/L	:	Gram per liter
GnRH	:	Gonadotropin Releasing Hormone
h(s)	:	Hour(s)
Hb	:	Haemoglobin

i.e.	:	That is
IM	:	Intramuscular
IU	:	International Unit
Kg	:	Kilogram
Mg	:	Milligrams
mg/kg	:	Milligram per kilogram
Ml	:	Milliliter
Mmol	:	Mili mole
mS	:	Mili-Siemens
N	:	Number
Ng	:	Nanogram
Nmol	:	Nano mole
ºC	:	Degree Celsius
OVS	:	Ovulation Synchronization
P	:	Phosphorus
P_4	:	Progesterone
PGF2α	:	Prostaglandin F2 alpha
PLT	:	Platelet
PMSG	:	Pregnant Mare Serum Gonadotropin
PO	:	Per os
PPA	:	Postpartum Anestrus
ppm	:	Parts per million
PR	:	Pregnancy rate
PRID	:	Progesterone Releasing Intravaginal Device
RBC	:	Red blood cell
Rpm	:	Rotation per min
SC	:	Subcutaneous
SE	:	Standard error
Spp.	:	Species
TEC	:	Total Erythrocyte Count
TLC	:	Total Leukocyte Count
Vs	:	Versus

Zn	:	Zinc
A	:	Alpha
Mg	:	Microgram
Ml	:	Microliter
Mmol	:	Micro mole
GPG+G	:	Ovsynch+GnRh
mcg	:	microgram
SS	:	Sex sorted
VER	:	Vaginal electrical resistance

INTRODUCTION

CHAPTER 1

INTRODUCTION

Since long ago, the animals have been an integral part of human life. With the progression of time, the dependency on domestic animals has increased. At the present time the human civilization cannot be imagined without an animal product we are using every day. Presently, huge dairy cattle numbers throughout the world is contributing in compensating the global food security. But, the rapid explosion of human population has increased the demand for animal products. So, researches are being targeted towards getting maximum production from the animals.

It is need of time to produce desired sex calf has been a long cherished dream of dairy cattle producers and scientists. Pre-conception selection of gender in bovines has lot of economic importance to justify its use as in most situations; one gender is more valuable (commercially) than other. Use of extensive modern techniques with mechanization for agricultural operations and laying a network of roads along with auto-revolution in the recent past is continuously taking away from male bovines of their traditional roles in crop husbandry, transportation and draught etc. Marketing, disposal or economic use of male calves continues to be a problem for the dairy farmers and planners. It leaves us in a peculiar situation where 50% population of the total progeny born is of unwanted sex (male) and remains more or less a liability rather than an actively performing asset. The sexed semen methodology used to minimize the numbers of the progeny belonging to the desirable and undesirable sex of calf. In certain situations, it may be desirable to get a bull calf from a particular excellent producing cow (elite dam); the sperm-sexing technology makes this option available.

The biotechnology for pre-selection of sex of the embryo is based on basic biological principles. It is non-invasive and doesn’t involve any foeticide. X and Y chromosomes is determined sex either female or male which is integral part of the genetic material. Female generally releases one ovum during each cycle. Ova are genetically similar as all ova possess one X as a sex chromosome. In contrast, spermatogenesis is a continuous process in male. A male liberates millions of sperm in each ejaculate. The sperm population is equally divided into two types. One category contains a single X as sex chromosome and the other category of sperm cells have a single Y as sex chromosome in addition to the full complement of autosomes. The Y chromosome is genetically dominant. Sex is determined solely by the presence or absence of the Y chromosome. The Y chromosome is smaller than X-chromosome and contains only a small number of genes essential for development of a fertile male. So a Y- bearing sperm has lesser DNA content (- 4%) than an X-bearing sperm.

The Sex Sorting laboratory has been recently established at BAIF, Uralikanchan, Rishikesh (Uttrakhand) by ST, Navasota and at Mehsana (Gujarat) by ABS. A few more such laboratories are expected to come up in the future. The technology has been commercially used in cattle for almost a decade and thousands of calves of desired gender have been born across the world. It has been learnt that a decade ago, Haryana Govt. imported 2000 straws of sex-sorted Holstein Friesian semen for a pilot study which used under the field conditions. The results have been encouraging in view of facts that it was the first experience, the test animals were spread throughout the state and thus reared under varying managemental conditions, in addition to varying skill levels of the inseminators ABS has started production of sex sorted semen of indigenous cattle and Buffaloes at its ultramodern facilities at Chitale Dairy Bhilwadi (Maharashtra).

In present study, the research is dealing with use of sex sorted semen for Artificial Insemination after synchronization of estrus in cows. Desired sex either female or male which is produced from semen having X or Y bearing sperm is known as sex sorted semen. Sex sorted semen increase the genetic progress in a herd by increasing the number of superior heifers and good male germ plasm from elite bulls which is used for future breeding program. The commercial sexed semen technique is now considered as most advance technique for milk and animal by-products production. The advancement of commercial sexed semen and its role in animal husbandry was summarized by (Garner and Seidel, 2008 Schenk *et al.*, 2009 and Kumar *et al.*, 2017).

Advantages of using sex sorted semen in dairy cattle

1. This also enables rapid herd expansion without the risk of introducing diseases that occur with purchased animals (Seidel, 1999).
2. Female to male ratio with 90:10 or vice-versa is ensured (Norman *et al*., 2010).
3. Reduced dystocia by preventing production of male calves (Fetrow *et al*., 2007).
4. The Production of superior bulls.
5. The cost of progeny testing programs is lowers and enhances the value of genetic markers of embryo transfer (De Vries *et al*., 2008).
6. Selective culling. By using sexed semen, selection intensity can be increased by choosing genetically superior dams of replacements which accelerate the rate of genetic gain in dairy herds (Weigel 2004, Khalajzadeh *et al.,*2012)
7. The principal benefit of using sexed semen is production of calves of desired sex (Seidel 2007, De Vrie *et al*., 2008)
8. It is possible to reduce the incidence of difficulty in first calvers (heifer calves are lighter than male calves) and additional replacement heifers for herd expansion may offer benefits in terms of improved biosecurity by increasing herd size while maintaining a closed herd (Weigel, 2004).
9. Sex-sorted semen can increase the profitability of dairy farms by increasing the number of genetically superior heifers born after AI (Hohenboken, 1999).

Many researchers successfully utilized this biotechnology by synchronizing the animals by using various estrus synchronization protocols. Synchronization of estrus can help in simplifying managemental practices. As in general, estrual cycicity means the rhythmic changes that occur in the reproductive system of a female animal starting from one estrus phase to another. Follicular and luteal phase are two phases of estrus cycle in cattle. Under follicular phase, follicle development is taken place. In luteal phase, formation and development of corpus luteum (CL) is taken place. The problem of anoestrus causes a huge economical loss to the farmers or producers. So, it needs to be solved immediately.

Kumar *et al*. (2014) have noted beautifully classified different ways of estrus induction. Various types of preparations are available in the market broadly divided into two types viz., non-hormonal and hormonal. Various hormones are being used for induction of estrus e.g. estrogen, progesterone, GnRH, prostaglandin, insulin, and anti-prolactin. The manipulation of the estrous cycle or induction of estrus brings a large percentage of a group of females into estrus at a short, predetermined time (Odde, 1990). Use of advanced managemental processes by which the managemental errors and its costs could be reduced is synchronization of estrus. To control the timing of the onset of estrus by controlling the length of the estrous cycle is the basic principle for the estrus synchronization. Different methods for inducing estrus are mentioned as follows:

1. Prostaglandin administration to regress the corpus luteum of the animal before the time of natural luteolysis.
2. Progesterone or synthetic progestin administration to suppress ovarian activity temporarily.
3. Creating estrous synchrony by using gonadotropin-releasing hormone or an analogue, which causes ovulation of a large follicle, helps in synchronizing estrous cycle in anestrous female.

Hence, the present research study is designed with the intention of comparative use of sex sorted and conventional semen in pubertal heifers and pleuriparous crossbred cows by using GPG+G protocol. Overall research study is targeted on following objectives.

Objectives:

1. To study effect of estrus induction protocol in pubertal heifers and pleuriparous crossbred cows.
2. To study conception rate using conventional and sex sorted semen in pubertal heifers and pleuriparous crossbred cows.

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

The present research work titled, **“Fertility response of the sex sorted semen in crossbred cattle’’** was planned to study the conception rate in crossbred cattle which will be a guide for utilization of a unique biotechnology with maximum benefits to the dairy farmers. It has also useful to increase productive and reproductive performance of dairy cattle. The literature pertaining to this study has been reviewed under following sub heads.

2.1 Efficacy of GPG+G protocol in heifers

2.1.1 Fertility response in estrus synchronized pubertal heifers with sex sorted semen

2.1.2 Fertility response in estrus synchronized pubertal heifers with conventional semen

2.2 Efficacy of GPG+G protocol in pluriparous cows

2.2.1 Fertility response in estrus synchronized pluriparopus cows with sex sorted semen

2.2.2 Fertility response in estrus synchronized pluriparopus cows with conventional semen

2.3 Estrual discharge properties at estrus and conception rate

2.4 Estrus detection by use of electronic heat detector

2.5 Duration of estrus

2.6 Estrus Synchronization response

2.7 Intensity of estrus

2.1 Efficacy of GPG+G protocol in heifers

The Ovsynch protocol is an effective breeding programme used in lactating dairy cows worldwide. The protocol involves the following three hormone administrations: a GnRH administration (GnRH-1) on day 0, followed by PGF2α administration on day 7, and another administration of GnRH (GnRH-2) 48 h later (Pursley *et al*., 1995). The administration of these hormones synchronizes the initiation of a follicular wave by ovulation of an ovulatory follicle, the regression of the corpus luteum and eventually, the ovulation of the dominant follicle of the new follicular wave. This precise synchronization of ovulation within an 8-h period, 24–32 h after the administration of GnRH-2, allows for successful timed artificial insemination (TAI) without any estrus detection. Therefore, the success of the Ovsynch protocol dramatically depends on the responses to these hormones. With respect to the response to GnRH administrations, fertility is better in cows that respond to the GnRH-1 in the Ovsynch protocol compared to those cows that do not respond (Vasconcelos *et al*., 1999; Moreira *et al*., 2001; Bello *et al*., 2006; Galvan and Santos, 2010; Keskin *et al*., 2010). Similarly, Galvao and Santos (2010) reported that any application that increases ovulation in response to GnRH-2 should result in increased fertility. For instance, administering exogenous progesterone (CIDR) between the GnRH-1 and PGF2α treatments is a modification of the Ovsynch protocol that is reported to increase the response to GnRH-2 and may therefore improve pregnancy rate (Stevenson *et al*., 2006; Galvao and Santos, 2010). However, another study showed that the administration of exogenous progesterone during the Ovsynch protocol decreases the response to GnRH-1 because of the possible negative feedback effect of progesterone on LH secretion (Galvao *et al*., 2004). Thus, any attempt to eliminate the possible negative effect on the response to GnRH-1 during progesterone administration, based on the Ovsynch protocol, may result in a higher response to GnRH administration and a higher pregnancy rate.

2.1.1 Fertility response in estrus synchronized pubertal heifers with sex sorted semen

Bodmer *et al.,* (2005) observed fertility in 132 heifers and cows after low dose insemination with sex-sorted and non-sorted sperm under field conditions. Heifers and cows were inseminated with 2x 10^6 x-bearing, frozen-thawed sperm (A) and 91 animals were inseminated with the same dose using non-sorted, frozen-thawed sperm (B). Pregnancy examination by ultrasound was performed twice, 30 to 40 days (PE1) and 70 to 90 days (PE2) after insemination. The pregnancy rates after PE1 were 33.3% (9/27) and 59.3% (16/27) in heifers (P = 0.05) and 27.6% (29/105) and 28.1% (18/64) in cows (P > 0.05) for groups A and B, respectively.

Cerchiaro *et al.,* (2007) assessed the fertility and purity of sexed semen used for inseminating Holstein-Friesian heifers in commercial dairy herds. Sex-sorted semen from 4 proven Holstein-Friesian bulls and available under commercial conditions was used on nulliparous Holstein heifers reared on 61 dairy farms of northern Italy. Data from 536 artificial inseminations with pregnancy diagnosis and 258 calvings were analyzed using the logistic regression procedure. The overall pregnancy rate for sexed semen was 51%.

Kurykin *et al*., (2007) studied objective to assess the effect on heifer pregnancy rate of deposition at three sites within the uterus of frozen-thawed sex sorted sperm at a fixed time after estrus synchronization. Estrus was synchronized in 209 heifers by administration of PGF2a 14 days apart. At 80–82 h after the second PGF2a injection, X-chromosomes bearing fractions of semen with 2.2 x 10^6 sperm in insemination dose were used for single insemination into the uterine body (UB-AI, n = 91) or for intracornual deposition in the middle of the uterine horn (MH-AI, n = 57) or close to the utero-tubal junction (UTJ-AI, n = 61). The overall pregnancy rate was 43.1%. Pregnancy rates did not differ (P > 0.05) among sites of sperm sperm deposition, Within UB-AI, MH-AI and UTJ-AI treatments, pregnancy rates were 41.8%, 49.1% and 39.3%, respectively (P > 0.05).

DeJarnette *et al.,* (2009) observed farm records of dairy herds (n = 211) using sexed semen. Sexed semen was predominantly used at first and second service in virgin heifers, which is reflected in younger ages at AI and at calving. Conception rates at first service averaged 47% for Holstein heifers and 53% for Jersey heifers, which were ~80% of that achieved with conventional semen. Analysis of inter-estrus intervals provides no evidence that cycle lengths are extended by use of sexed semen.

DeJarnette *et al*., (2010) observed effects of 2.1 and 3.5 × 106 sex-sorted sperm dosages on conception rates of Holstein cows and heifers. Among heifers, 6,268 services were retrieved from 45 herds (298 ± 4.2 services/ sperm dose per sire; range: 244 to 344). Conception rate of heifers was influenced by the sire by treatment interaction. Conception rate of the 2.1 and 3.5 × 106 sex-sorted sperm dosages were comparable in 6 of 7 sires. Conception rate of both sex-sorted dosages were less than those of conventional semen for 6 of 7 sires. Across sires, heifer conception rates for 2.1 and 3.5 × 106 sex-sorted sperm dosages and 15 × 106 conventional dosages were 44, 46, and 61%, respectively.

Filhoa *et al*., (2010) studied strategies to improve pregnancy per insemination using sex-sorted semen in dairy heifers detected in estrus. The objective was to improve pregnancy per artificial insemination (P/AI; 35–42 d after AI) in virgin Jersey heifers bred by AI of sex-sorted semen after being detected in estrus. Giving 100 micro gram of GnRH at first detection of estrus, with AI 12 h later, did not affect P/AI in Experiment I GnRH 47.2% vs. No GnRH 51.7%; or Experiment II GnRH 53.1% vs. No GnRH 48.6%. The P/AI of heifers inseminated from 12 to 16 h after the onset of estrus (37.7%) was less than those inseminated from 16.1 to 20 h (51.8%), and 20.1 to 24 h (55.6%).

Chebel *et al*., (2010) studied sex-sorted semen for dairy heifers and its effects on reproductive and lactational performances. Holstein heifers (herd A = 227 and herd B = 1,144) received first artificial insemination (AI) with sex-sorted semen (SX; n = 343) or conventional semen (CS; n = 1,028), and all heifers that displayed estrus after first AI were reinseminated with conventional semen up to 11 times before being culled. Pregnancy per AI after first AI was greater for CS heifers than for SX heifers (51.8 vs. 40.2%).

DeJarnette *et al*., (2011) observed conception rates of Holstein heifers after AI with 2.1 or 10 × 106 sperm dosages of sex-sorted or conventionally processed sperm were compared. Ejaculates collected by artificial vagina from 8 Holstein sires were cryopreserved at either 2.1 or 10 × 106 sperm per dose with or without sorting to 90% purity for X-chromosome-bearing spermatozoa using flow cy-tometry. Across herds and sires, the conception rates of each semen type by sperm dosage combination were as follows: 2.1 × 106 sex-sorted, 38%, n = 2,319; 10 × 106 sex-sorted, 44%, n = 2,279; 2.1 × 106 conventional, 55%, n = 2,282; and 10 × 106 conventional, 60%, n = 2,292.

Sales *et al*., (2011) evaluated the effects of timing of insemination and type of semen in cattle subjected to timed artificial insemination (TAI). There was an interaction ($P \leq 0.06$) between time of AI and type of semen on pregnancy per AI (P/AI, at 30 to 42 d after TAI); it was greater when sex-sorted sperm ($P \geq 0.01$) was used at 60 h (31.4%; 32/102) than at 54 h (16.2%; 17/105). The P/AI was greatest (37.9%) for TAI performed between 0 and 12 h before ovulation, whereas P/AI was significantly less for TAI performed between 12.1 and 24 h (19.4%) or 24 h (5.8%) before ovulation. In conclusion, sex-sorted sperm resulted in a lesser P/AI than non-sorted sperm following TAI.

Funston and Meyer (2012) studied the objective to evaluate the use of sexed semen in a commercial heifer development program. Heifers (n = 500) were fed 0.5 mg/d of melengestrol acetate per animal for 14 d and 19 d later, administered $PGF2\alpha$ (PGF), and Estrotect heat detection patches were placed on their tail heads. Following PGF injection, heifers were detected for standing estrus and AI approximately 18 to 24 h following detection of standing estrus. Heifers not detected in estrus by 72 h after PGF injection were time inseminated (TAI) and given a GnRH injection 77 to 78 h post-PGF injection. Heifers were AI with one of 2 sires, either conventional or sexed semen, creating 4 possibilities for AI sire. At each AI session, heifers were divided evenly to receive either sexed or conventional semen from the same sires. Pregnancy rate was greater ($P < 0.01$) for heifers inseminated with conventional compared with sexed semen (58.4 vs. 41.0%). In addition, more ($P < 0.01$) heifers detected in standing estrus were pregnant (≥55.9%) than heifers TAI (24.0%).

Hutchinson *et al*., (2013) studied the role of sexed semen use in virgin heifers and lactating cows. Sexed frozen-thawed semen used in lactating cows for the first 3 wk of the breeding season (SFro1), sexed fresh semen used in lactating cows for the first 6 wk of the breeding season (SFre2), or sexed frozen-thawed semen used in lactating cows for the first 6 wk of the breeding season (SFro2). In the SFro1, SFre1, SFro2, and SFre2 herds, sexed semen was used for the first and second artificial insemination in virgin heifers. Pregnancy rates achieved with sexed fresh and sexed frozen-thawed semen was assumed to be 94 and 75% of those achieved with conventional frozen-thawed semen, respectively.

Mallory *et al*., (2013) studied pregnancy (P/AI) with sex sorted (SS) and conventional semen (CON) from a single sire within a fixed time A.I. (FTAI) programme designed for dairy heifers. Holstein heifers (n =240) were assigned to treatment (CON or SS) according to body weight and reproductive tract score. Estrous response did not differ between CON (63/120; 53%) and SS (70/120; 58%) treatments. The CON heifers, however, achieved greater FTAI P/AI (82/120; 68%) compared with SS (45/120; 38%) heifers.

Healy *et al*., (2013) investigated conception rates and other reproductive outcomes achieved with artificial insemination (AI) of nulliparous Holstein heifers using sexed and conventional semen in a commercial Australian dairy herd in central western New South Wales. Sexed semen was primarily used at first and second service. Empirical conception rates of 31.6 and 39.6% were achieved for sexed and unsexed semen respectively.

Abdalla *et al*., (2014) studied the fertility of commercially available sexed semen and to economically analyse three assumed strategies for its application in Holstein heifers. In the first part of the study, a total of 426 heifers were inseminated with sexed semen from 7 bulls and 325 heifers were inseminated with unsexed semen from 5 bulls. The pregnancy at 40 and 90 days post insemination were calculated. Heifers inseminated with sexed semen had significantly lower ($P<0.001$) pregnancy-

40, pregnancy- 90 and calving rates (34, 32.2 and 29.3%; respectively) than those inseminated with unsexed semen (62.5, 57.8 and 51.1%; respectively).

Noonan *et al.,* (2016) studied factors associated with fertility of nulliparous dairy heifers following a 10-day fixed-time artificial insemination program with sex-sorted and conventional semen. Weight, age, expression of oestrus, sire, semen type (frozen sex sorted or frozen conventional) and timing of insemination were examined for their relationship with first-service conception rates. First-service conception rates were 40.3% and 56.0% for sex-sorted and conventional semen, respectively, and were significantly higher when oestrus was expressed.

Lenz *et al*., (2016)mentioned the gap in fertility between conventional and sex-sorted bovine sperm, known to be on the order of 10 percentage points, has never been bridged, even by increasing the number of sex-sorted sperm per inseminate. A concerted effort in the last few years has resulted in substantial changes in all stages of the sex-sorting process to develop an improved sex-sorted product called SexedULTRA™ (Sexing Technologies, Navasota, TX, USA). A total of 6,930 Holstein heifers were inseminated across 41 commercial herds in the United States. Sex-sorted bull sperm following the SexedULTRA™ method resulted in a greater ($P < 0.001$) conception rate compared with the XY method sperm (45.7 ± 1.7 v. 41.2 ± 1.6%). This is the first report of an improvement in conception rates using sex-sorted bovine semen in a decade since it became commercially available.

Murphy *et al.*, (2016) studied the role of sexed semen within alternative breeding strategies to determine the effects of sexed semen use in heifers and lactating cows on replacement heifer numbers and rate of herd expansion in a seasonal dairy production system. Each AI protocol was assessed under 3 scenarios of sexed semen conception rate (SS-CR): 100, 94, and 87% relative to that of conventional semen.

Thomas *et al*., (2017) evaluated the relative fertility of sexed ULTRATM sex-sorted semen compared to conventional, non-sex-sorted semen when used among beef heifers in conjunction with split-time AI following the 14-d CIDR-PG protocol. Estrus detection aids were applied at PG on Day 30 to evaluate estrous response rate, and split-time AI was performed based on estrous response. At 66 h after PG (Day 33), heifers having expressed estrus received timed AI. Pregnancy rates to AI across locations tended to be higher for heifers inseminated with conventional semen (60%) compared to sex-sorted semen (52%).

Colazo and Mapletoft, (2017) studied comparative pregnancy per AI (P/AI) of heifers inseminated with sex-selected or conventional semen after estrus detection (ED) or timed-AI (TAI). Heifers in the ED group received 2 treatments with prostaglandin F2a 14 d apart and those in the TAI group received a modified 5-day Co-synch protocol plus intravaginal progesterone releasing insert device (PRID) and were inseminated 72 h after PRID removal. Overall P/AI were 69.2% (74/107) and 64.1% (75/117) for conventional and sex-selected semen (P. 0.05).

Guner *et al*., (2020) observed effect of delaying the time of insemination with sex-sorted semen on pregnancy rate in Holstein heifers. Heifers in oestrus were detected and inseminated only by using heat–rumination neck collar comprised electronic identification tag at the age of 13–14 months. Heifers (n = 283) were randomly assigned to one of three groups according to the timing of insemination at 12–16 hr (G1, n = 97), at 16.1–20 hr (G2, n = 94) and at 20.1-24 hr (G3, n = 92) after reaching the activity threshold. The mean duration of oestrus was 18.6 ± 0.1 hr, and mean peak activity was found at 7.5 ± 0.1 hr after activity threshold. The mean interval from activity threshold to ovulation was 29.4 ± 0.4 hr. The overall pregnancy per AI (P/AI) was 53.0% at 29-35 days and 50.9% at 60-66 days after AI.

Chebel and Cunha (2020) tried optimization of timing of insemination of dairy heifers inseminated with sex-sorted semen. Holstein heifers (n = 1,207) were fitted with a collar containing an automated estrus-detection device (HRLDn tags, SCR Engineers Ltd., Netanya, Israel) at 10.7} 0.02 mo of age. Once they reached 330 kg, heifers were enrolled in an ovulation synchronization protocol (5-d Cosynch + controlled internal drug release; Holstein heifers (n = 1,207) were fitted with a collar containing an automated estrus-detection device (HRLDn tags, SCR Engineers Ltd., Netanya, Israel) at 10.7 ± 0.02 mo of age. Once they reached 330 kg, heifers were enrolled in an ovulation synchronization protocol (5-d Cosynch + controlled internal drug release; ± 2.6%) compared with SS Early (43.3 ± 2.6%) and SS Late (44.8 ± 2.7%).

Zargaran *et al.,* (2021) studied reproductive performance of Holstein Heifers inseminated with sex sorted semen in various herd sizes. Evaluated reproductive traits were conception rate, dystocia, stillbirth, abortion as well as days open and days from calving to the first service in heifers inseminated with various semen types and various farm sizes. The result illustrated that using sex-sorted semen dramatically decreases the proportion of reproductive disorders, yet it led to lower conception rate.

Guner *et al.,* (2021) compared pregnancy per AI of heifers inseminated with sex-sorted or conventional semen after oestrus detection or timed artificial insemination. Holstein heifers were randomly assigned to one of the following treatments in a 2 × 2 factorial design. Heifers in the EST group were inseminated with sex-sorted (n = 114) or conventional semen (n = 100) after spontaneous or induced oestrus. Heifers in the TAI, subjected to the 5-day Cosynch+Progesterone protocol (GnRH+P4 insertion-5d- PGF2α+ P4 removal-1d- PGF2α -2d- GnRH+ TAI), were inseminated with sex-sorted (n = 113) or conventional semen (n = 88). Overall P/AI was 60.7% for EST and 54.2% for TAI regardless of types of semen and 68.1% for conventional and 48.9% for sex-sorted semen regardless of insemination strategies. Fertility of heifers inseminated with either sex-sorted (53.5%; 44.2%) or conventional (69.0%; 67.0%) semen did not differ between EST and TAI respectively.

2.1.2 Fertility response in estrus synchronized pubertal heifers with conventional semen

Pursley *et al*., (1995) evaluated Pregnancy Rates per Artificial Insemination for Cows and Heifers Inseminated at a synchronized ovulation or synchronized Estrus. Treated cows and heifers received a protocol that used GnRH and PGF2a to synchronize ovulation (Ovsynch). Cows and heifers that were treated with Ovsynch were injected i.m. with 100 mg of GnRH at a random stage of the estrous cycle. Seven days later, cows and heifers in this group received 25 mg of PGF2a, followed by a second injection of 100 mg of GnRH 30 to 36 h later. Subsequently, the treated cows and heifers received AI 16 to 20 h after the second injection of GnRH. Pregnancy rates per AI were similar (38.9% vs. 37.8%) for control cows and cows treated with the Ovsynch protocol, respectively. However, pregnancy rate per AI was greater for control heifers (74.4%) than for heifers treated with Ovsynch (35.1%).

Pursley *et al.*, (1997) noted that a reduction in pregnancy rates of dairy heifers submitted to the Ovsynch/TAI protocol was detected when the program was compared to pregnancy rates obtained after three consecutive synchronization treatments with PGF2α. Such results could be due to the fact that estrus detection is higher and fertility is greater in dairy heifers than in lactating cows. Nonetheless, if pregnancy rates obtained after the initial synchronization with PGF2α (28.2%) were compared to pregnancy rates obtained by the Ov-synch/TAI service (35.1%), there would be difference between the two groups.

Xu and Burton (1999) observed the reproductive performance of heifers after estrus synchronization and fixed-time AI was compared with non synchronized heifers in 25 spring-calving herds. Heifers in the synchronized group were treated with a combination of progesterone, estradiol benzoate, and PGF2a and were inseminated between 50 and 54 h after progesterone treatment. The conception rate of synchronized heifers to the fixed-time AI (53.2%) and to AI after resynchronization (53.1%) was lower than that of control heifers (63.7%).

Stevenson *et al*., (1999) studied reproductive outcomes for dairy heifers treated with combinations of PGF2*α*, norgestomet, and gonadotropin-releasing hormone. They conducted three experiments to test various protocols for synchronizing estrus, ovulation, or both before insemination of heifers. The ovsynch protocol produced results inferior to those obtained with Select Synch and a standard PGF2*α* protocol that allowed insemination at detected estrus after one or two injections plus timed inseminations at 72 to 80 h in heifers failing to be detected in estrus after both injections. The Ovsynch protocol produced pregnancy rates exceeding 40%.

Moreira *et al*., (2000) studied effect of day of the estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers.

Cycling Holstein heifers (n = 24) were injected twice with prostaglandin F2*α* to induce estrus and were scanned by ovarian ultrasonography to determine the day of ovulation (d 0).

Richardson *et al*., (2002a) studied characteristics of estrus before and after first insemination and fertility of heifers after synchronized estrus using GnRH, PGF2*α*, and progesterone. Their objectives were to determine fertility of heifers after synchronization of estrus using PGF2*α*, preceded by progesterone (P4), GnRH, or both, and to examine the variability of estrual characteristics in heifers before first and second AI. In dairy heifers, conception and pregnancy rates were greatest in the P4+PGF treatment and least in the GnRH+PGF treatment, whereas those in the P4+GnRH+PGF treatment were intermediate.

Yilmaz *et al*., (2011) studied effect of PGF2α and GnRH injections applied before ovsynch on pregnancy rates in cows and heifers. The objective of the study was to investigate the effect of PGF2α and GnRH injections applied before Ovsynch protocol (G6G) on pregnancy rates in cows and heifers. Totally, 196 Holstein cows in postpartum 50-100 days and 169 Holstein heifers were used. Pregnancy rates in cows and heifers were detected 57.50-60.00%; 37.83-32.50% and 53.78-59.18% in 1st, 2nd and 3rd groups, respectively.

Lima *et al*., (2011).performed two experiments evaluated the effects of the first GnRH injection of the 5-d timed artificial insemination (AI) program on ovarian responses and pregnancy per AI (P/AI), and the effect of timing of the final GnRH to induce ovulation relative to AI on P/AI. In experiment 1, 605 Holstein heifers were synchronized for their second insemination and assigned randomly to receive GnRH on study d 0 (n = 298) or to remain as untreated controls (n = 307). Pregnancy per AI was not affected by treatment at d 32 or 60 (GnRH = 52.5 and 49.8% vs. control = 54.1 and 50.0%).In experiment 2, 1,295 heifers were synchronized for their first insemination and assigned randomly to receive a CIDR on d 0, PGF2αand removal of the CIDR on d 5, and either GnRH 56h after PGF2α and AI 16 h later (OVS56, n = 644) or GnRH concurrent with AI 72 h after PGF2α (COS72; n = 651).

Estrus at AI was greater for COS72 than for OVS56 (61.4 vs. 47.5). Treatment did not affect P/AI on d 32 in heifers displaying signs of estrus at AI, but COS72 improved P/AI compared with OVS56 (55.0 vs. 47.6%) in those not in estrus at AI.

Thomas *et al.*, (2014a) designed two experiments to test the hypothesis that pregnancy rates after fixed-time artificial insemination (FTAI) in beef heifers and cows may be improved by delaying insemination of females that have not expressed estrus before FTAI. In Exp. 1, estrus was synchronized for 931 heifers across 3 locations using the 14-d CIDR-PG protocol (controlled internal drug-release [CIDR] insert [1.38 gm progesterone] on d 0 with removal of CIDR insert on d 14;25 mg PGF2α 16 d after CIDR insert removal on d 30; and 100 μg GnRH on d 33, 66 h after PGF2α). Estrous detection aids (Estrotect) were applied at PGF2α on d 30, and estrous expression was recorded at GnRH on d 33.Heifers within each location were randomly assigned to 1 of 2 treatments based on weight and reproductive tract score (RTS): 1) FTAI (concurrent with GnRH, 66 h after PGF2α) regardless of estrous expression or 2) FTAI for heifers expressing estrus and delayed AI (20 h after GnRH) for heifers failing to express estrus. Heifers assigned to treatment 2 achieved a higher AI pregnancy rate than heifers assigned to treatment 1 (54 versus 46%;P = 0.01).

Silva *et al.*, (2015) evaluated the effects of synchronizing estrus and ovulation to implement a timed artificial insemination (AI) at first insemination on reproductive performance and cost per pregnancy in dairy heifers. Six hundred eleven Holsteins heifers at approximately 400 d of age from 3 farms were enrolled in the study. Six days before moving to the breeding pens, heifers were allocated randomly to AI after detected estrus from study d 0 to 84 (CON, n = 306), or to timed AI for first AI followed by detected estrus for the remainder of the 84-d study (TAI, n = 305). Heifers receiving TAI were enrolled in the 5-d timed AI protocol on study d −6 (d −6, GnRH and a progesterone insert; d −1, PGF2α and insert removal; d 0, PGF2α; d 2, GnRH + AI), and they were allowed to be bred the day before scheduled timed AI if detected in estrus.

Pregnancy at first AI did not differ between treatments (CON = 58.3 vs. TAI = 62.8%). In contrast, TAI increased pregnancy per AI (P/AI) compared with CON in heifers inseminated with sex-sorted semen (CON = 31.6 vs. TAI = 54.8%).

2.2 Efficacy of GPG+G protocol in pluriparous cows

The GnRH is a naturally occurring hormone that stimulates the release of FSH and LH by the anterior pituitary gland. These two hormones act on the ovary, stimulating follicular development (FSH) and ovulation (LH). The use of GnRH has been associated to that of prostaglandin, in order to decrease the variation in the ovulation time after prostaglandin treatment. One program that has been extremely successful for insemination of cows at a fixed time without the need for detection of estrus is the Ovsynch program. Ovulation synchronization can be obtained using GnRH + prostaglandin after 7 days + GnRH after 48hrs. This system synchronizes follicle maturation with regression of the corpus luteum before the GnRH-induced ovulation and timed insemination. The animals are inseminated 16–20 h after the second injection of GnRH (Barile, 2012). Synchronization of estrus and fertility with a combination of GnRH and PGF2α are good for cyclic females and this combination may induce cyclicity in cows experiencing postpartum anoestrus (Pursley *et al*., 1995).

2.2.1 Fertility response in estrus synchronized pluriparous cows with sex sorted semen

Doyle *et al.* (1999) implemented synchronization program in Angus cows by using GnRH and PGF2α treatments. Cow received half of 1×10^6 frozen- thawed sorted sperms and few cows received half of 5×10^5 cooled-to-18°C sorted sperm deposited into each uterine horn 6 to 26 h after observed estrus. The pregnancy rates recorded were 23 % and 25 % respectively.

Sales *et al*., (2011) studied timing of insemination and fertility in dairy and beef cattle receiving timed artificial insemination using sex-sorted sperm. The objective was to evaluate the effects of timing of insemination and type of semen in cattle subjected to timed artificial insemination (TAI). In Experiment 1, 420 cyclic Jersey heifers were bred at either 54 or 60 h after P4-device removal, using either sex-sorted (2.1 x 10^6 sperm/straw) or non-sorted sperm (20 x 10^6 sperm/straw). There was an interaction (P= 0.06) between time of AI and type of semen on pregnancy per AI (P/AI, at 30 to 42 d after TAI); it was greater when sex-sorted sperm (P<0.01) was used at 60 h (31.4%; 32/102) than at 54 h (16.2%; 17/105). In contrast, altering the timing of AI did not affect conception results with non-sorted sperm (54 h50.5%; 51/101 versus 60 h51.8%; 58/112; P= 0.95).

Sá Filho *et al*., (2012) used sex-sorted sperm in timed artificial insemination programs for suckled beef cows. Three experiments were designed to evaluate methods to optimize the use of sex-sorted sperm in timed AI (TAI) programs for suckled beef cows. In all 3 experiments, suckled Bos indicus cows were synchronized using an intravaginal progesterone (P4) device during 8 d and a 2.0-mg injection of intramuscular estradiol benzoate (EB) at device insertion. The females received PG and eCG (300 IU) at P4 device removal and 1.0 mg of EB 24 h later. The cows were inseminated 60 to 64 h after P4 device withdrawal There were effects of the occurrence of estrus (P = 0.0003) and the type of sperm (P = 0.05) on pregnancy per AI [P/AI; no estrus, non-sex-sorted = 43.6% (27/62); estrus, non-sex-sorted = 58.5%; (107/183); no estrus, sex-sorted = 33.9% (21/62), and estrus, sex-sorted = 50.0% (92/184)]

Khalajzadeh *et al*., (2012) studied Stochastic simulation used for studying the impacts of sexed semen on genetic progress and reproductive performance of dairy cows. Three strategies were compared: WSS (use unsexed semen in cows and heifers), SSH (use sexed semen in heifers and unsexed semen in cows) and SSCH (use sexed semen in both cows and heifers). Conception rate (CR) of unsexed semen was considered to be 35% and 65% in cows and heifers, respectively. CR of sexed semen was considered to be 15 (20% in cows and 50% in heifers), 10, 5 and 0 percentage points lower than unsexed semen.

Sá Filho *et al*., (2013) studied use of sex-sorted sperm in lactating dairy cows upon estrus detection or following timed artificial insemination. They evaluated the use of sex-sorted sperm upon estrus detection (ED) or following timed artificial insemination (TAI) in lactating dairy cows. Additionally, the effect of the presence of a corpus luteum (CL) at the beginning of the TAI protocol was verified. Cows (539 crossbred Gir Holstein and 87 Holstein) were classified according to the presence or absence of CL by ultrasonography exam. However, cows receiving AI upon ED (CL-ED/AI = 31.7%, 32/101) presented higher (P = 0.03) pregnancy per AI (P/AI) than cows bred following TAI (CL-TAI = 19.4%, 35/180 and No CL-TAI = 23.9%, 49/205).

Mellado *et al*., (2014) observed effects of month of breeding on reproductive efficiency of Holstein cows and heifers inseminated with sex-sorted or conventional semen in a hot environment. The main objective of this study was to assess the effect of month of breeding on reproduction performance of Holstein heifers and cows inseminated with sex-sorted or conventional semen in a hot environment. Pregnancy per artificial insemination (P/AI; 64,666 services over an 8-year period) both in heifers (n =22,313) and cows (n =42,353) from a large dairy herd in northern Mexico (26°N) were evaluated with the GENMOD procedure of SAS, with respect to month of AI. Overall, P/AI with sex-sorted semen was greater (P <0.01) in heifers (41.6 %) than cows (17.3%). P/AI for cows serviced with conventional semen was 10 % points higher (P <0.01) in January and December (31 vs. 21 %) than cows serviced with sex sorted semen. While there was no difference in P/AI between the sex-sorted sperm and conventional semen in cows inseminated in July (16 and 18 %, respectively), P/AI plummeted for both groups of cows during the summer and fall (more severe heat stress).

Xu (2014) observed application of liquid semen technology improves conception rate of sex-sorted semen in lactating dairy cows. The objective was to compare reproductive performance of liquid sex-sorted (SS) semen with that of conventional (CON) semen in lactating dairy cows. Between 2011 and 2013, commercial dairy herds (n =101, 203, and 253 for 2011, 2012, and 2013, respectively) with predominantly Holstein-Friesian cows were enrolled in a contract mating program to produce surplus heifers for export using liquid SS semen. liquid SS semen only required half the dose rate of frozen SS semen to achieve a reproductive performance of over 94% of CON semen in lactating dairy cows. Careful planning and a robust distribution network are required to avoid semen wastage and to maximize the benefit of liquid SS semen.

Karakaya *et al.,* (2014) conducted study on total 148 cyclical cows synchronized with ovsynch protocol. Cows were inseminated with frozen-thawed sex sorted semen. Pregnancy per AI on day 31 tended for sorted semen was 31.8 % whereas P/AI on day 62 was 25.7 %.

Thomas *et al.,* (2014b) tested the hypothesis that delayed insemination of non estrous cows would increase pregnancy rates when using sex-sorted semen in conjunction with fixed time artificial insemination (FTAI). Estrus was synchronized for 656 suckled beef cows with the 7-d CO-Synch + controlled internal drug release (CIDR) protocol (100 mcg GnRH + CIDR [1.38 g progesterone] on d 0, 25 mg PGF2α at CIDR removal on d 7 and 100mcg GnRH d 10, 66 h after CIDR removal). Pregnancy rates achieved with sex-sorted semen in Treatments 1 and 2 were recorded as 51 % and 42 %, respectively among cows that expressed estrus.

Bombardelli *et al*., (2016) studied the objective of this observational experiment was to determine the association between the interval from reaching activity threshold (AT) to artificial insemination (AI) with sex-sorted semen and the probability of pregnancy. Jersey cows (n = 678) from a commercial dairy herd. There was a quadratic effect of the interval between reaching AT and AI on probabilities of pregnancy at 31 ± 3 (P = 0.07) and 66 ± 3 (P = 0.15) days after AI. Pregnancy per AI at 66 ± 3 days after AI was higher for cows inseminated between 23 and 41 hours after the onset of estrus (≤3 hours = 20.0%, 4–12 hours = 27.1%, 13–22 hours = 39.1%, 23–41 hours = 45.6%, and ≥42 = 40.0%).

Hall *et al*., (2017) studied impact of delayed insemination on pregnancy rates to gender selected semen in a fixed-time AI system. The objectives of the current experiment were to determine if delaying insemination by 8 h in a FTAI protocol would alter estrus expression and pregnancy rates in cows inseminated with sex-sorted semen, Over three breeding seasons, postpartum cows (n = 839) were estrous synchronized using the 5-day CO-Synch + CIDR system. Cows were given GnRH (100 μg i.m., Factrel) at time of insertion of a controlled internal drug releasing device (CIDR; There was no difference (P > 0.05) in pregnancy rates to sex-sorted semen or final pregnancy rates between NORM and DELAY cows. Pregnancy to sex-sorted semen averaged 35.2% whereas final pregnancy rates were 90.6%.

Silva *et al*., (2018) conducted two experiments to assess a hormonal strategy developed to reduce animal handling for timed artificial insemination (TAI) with sex-sorted semen. Four-hundred ninety-one (491) suckled beef cows received a progesterone (P4) intravaginal device and 2 mg intramuscular (im) injection of estradiol benzoate (EB) on a randomly chosen day of the estrus cycle (Day 0) in Experiment 1. Cows were treated with 500 μg of sodic cloprostenol (PGF2α) and with 300 IU of eCG at P4 device removal (Day 8); these cows were also randomly assigned to receive 1 mg of estradiol cypionate (EC) administered at P4 device removal (treatment EC-0h) or 1 mg of EB 24 h after P4 device removal (treatment EB-24h). Both treatments were timed inseminated (TAI) with sex-sorted semen 60 h after P4 device removal. Cows treated with EC-0h presented higher pregnancy rate per AI (P/AI) [45.0% (113/251)] than the ones treated with EB-24h [35.4% (85/240); P = 0.03)].

Sharma *et al*., (2018) undertaken a study in the Lohaghat block of Champawat district, Uttarakhand to assess the conception rate and sorting efficiency of sexed semen under field condition. AI was done in 218 animals during the study period. Cows were divided in two groups G1 and G2 on the basis of use of sexed and unsexed semen, respectively for AI. In G1, total 70 cows inseminated with sexed semen from three centers of Lohaghat block and conception rate, female calves and male calves were 40%, 82.14% and 17.85%, respectively. Total 148 cows were inseminated with unsexed semen from the same three centers. The observed conception rate, female calves and male calves were 49.32%, 50.68% and 49.31%, respectively.

Crites *et al.*, (2018) recorded conception risk of beef cattle after fixed-time artificial insemination using either sexed ultra™ 4m sex-sorted semen or conventional semen. The objective of this study was to compare conception rates of female beef cattle inseminated at a fixed time with either conventional (CON) or SexedUltra™ sex-sorted (SU) semen. Treatments included CON or SU with two sires represented within each treatment. Cows (n ¼ 316) and heifers (n ¼ 78) from six locations were randomly assigned treatment. Estrotect™ estrous detection aids were applied at CIDR removal and patch activation was recorded at insemination. Conception rate for estrual females (62.8%) was greater (p¼0.0001) than non-estrual females (38.7%) at FTAI regardless of treatment. Furthermore, the conception rates were similar (P ¼ 0.61) between conventional (61.9%) and sex-sorted semen (63.8%) when estrus was expressed prior to FTAI.

Karakaya-bilen *et al.,* (2019) conducted study on pregnancy per AI when cows were subjected to ovsynch protocol. The cows received an injection of GnRH on experiment Day -10 (10 μg, i.m. buserelin acetate; Receptal® Intervet, Turkey) followed 7 days later by an injection of PGF2α on experiment Day -3 (500 μg i.m. cloprostenol; Estrumate® CEVA-DIF, Turkey), and a final dose of GnRH administered 56 h after PGF2α. The Cows were inseminated with sex sorted semen (SS) containing 2.2 x 106 morphologically normal and 1.9 x 10^6 progressively motile sperm cells/straw. Conception rate was recorded as 38.1 when inseminated with gender sorted semen on day 31.

Maicas *et al.*, (2019) studied fertility of fresh and frozen sex-sorted semen in dairy cows and heifers in seasonal-calving pasture-based herds. Objective of the study was to evaluate the reproductive performance of dairy heifers and cows inseminated with fresh or frozen sex-sorted semen (SS) in seasonal-calving pasture-based dairy herds. Ejaculates of 10 Holstein-Friesian bulls were split and processed to provide (1) fresh conventional semen at 3×10^6 sperm per straw (CONV); (2) fresh SS at 1×10^6 sperm per straw (SS-1M); (3) fresh SS semen at 2×10^6 sperm per straw (SS-2M); and (4) frozen SS at 2×10^6 sperm per straw (SS-FRZ). In heifers, P/AI was greater for inseminations with CONV (60.9%) than with SS-FRZ (52.8%)

but did not differ from SS-1M (54.2%) or SS- 2M (53.5%). Cows inseminated with CONV had greater P/AI (48.0%) than cows inseminated with SS, irrespective of treatment.

Thomas *et al*., (2019) performed an experiment designed to compare fertility of SexedULTRA 4M™ sex-sorted semen and conventional, non-sex-sorted semen following either fixed-time artificial insemination (FTAI) or split-time artificial insemination (STAI) of mature suckled beef cows. Units of sex-sorted and conventional semen were produced using contemporaneous ejaculates from three commercially available sires. Units of conventional semen were generated with 25.0×10^6 live cells per 0.25 ml straw prior to freezing, and units of sex-sorted semen were generated using the Sexed ULTRATM Genesis III sorting technology with 4.0×10^6 live cells per 0.25 ml straw prior to freezing. Sex-sorted units were sorted to contain X chromosome-bearing sperm cells at an accuracy level of >90%. Cows (n = 1620) across four herds were treated with the 7-d CO-Synch + CIDR protocol [administration of gonadotropin-releasing hormone (GnRH) and insertion of a progesterone insert (CIDR) on Day -10, followed by administration of prostaglandin F2α (PG) and removal of CIDR inserts on Day -3]. Pregnancy rates to AI were affected ($P = 0.04$) by the interaction of bull and semen type. Greater pregnancy rates were obtained with conventional semen versus Sexed ULTRA 4M™ sex-sorted semen when using semen from Bull A (64% [176/277] versus 36% [100/278]; $P < 0.0001$) and Bull B (72% [200/277] versus 57% [156/276]; $P < 0.01$), whereas pregnancy rates to AI did not differ between conventional and Sexed ULTRA 4M™ sex-sorted semen when using semen from Bull C (58% [149/258] versus 52% [131/254]).

Maicas *et al*., (2020) evaluated the reproductive per-formance of frozen sex-sorted sperm at 4×10^6 sperm per dose (SexedULTRA 4M, Sexing Technologies, Na-vasota, TX) relative to frozen conventional sperm in seasonal-calving pasture-based dairy cows. Pregnancy diagnosis was performed by ultrasound scanning (n = 7,246 records available for analysis). Generalized linear mixed models were used to examine effects on preg-nancy per AI (P/AI) at first artificial insemination, with sperm treatment (CONV vs. SS), bull (n = 10), and treatment × bull interaction as the fixed effects, and herd (n = 142) as a random effect. Overall, P/AI was greater for cows inseminated with CONV than for those inseminated with SS (59.9% vs. 45.5%; 76.0% relative to CONV).

Dawod and Elbaz (2020) executed the research work to evaluate the fertility of sexed semen compared with conventional semen with regard to the puberty and breeding ages of Holstein dairy heifers subjected to double ovsynch protocol with FTAI. A total of 468 Holstein heifers were divided into two groups. All heifers received the Ovsynch-56 TAI protocol: GnRH followed by PGF2α injection 7 days later, GnRH 56 h after PGF2α, and TAI 12 to 14 h later. A total 346 heifers were inseminated with sex sorted semen. Estrus was synchronized using the double ovsynch protocol. Numbers were estimated for pregnancy at 40 and 60 days post insemination, embryonic loss, and abortion. The results revealed that the heifers inseminated with sexed semen had (51.45%) pregnancy rate in animals attempted during first service.

Drake *et al*., (2020) studied the objective to use ovulation synchronization with timed artificial insemination (TAI) to evaluate the effect of timing of artificial insemination (AI) with frozen sex-sorted sperm on fertility performance in pasture-based compact calving herds. Ejaculates from 3 Holstein-Friesian bulls were split and processed to provide frozen sex-sorted sperm (SS) at 4×10^6 sperm per straw, and frozen conventional sperm at 15×10^6 sperm per straw (CONV). A modified Progesterone-Ovsynch protocol was used for estrous synchronization, with TAI occurring 16 h after the second GnRH injection for cows assigned to CONV, and either 16 h (SS-16) or 22 h (SS-22) for cows assigned to SS. Pregnancy diagnosis was conducted by transrectal ultrasound scanning of the uterus 35 to 40 d after TAI (n = 2,175 records available for analysis). Pregnancy per AI was greater for CONV compared with both SS-16 and SS-22 (61.1%, 49.0%, and 51.3%, respectively), and the SS treatments did not differ from each other (relative P/AI for SS-16 and SS-22 vs. CONV were 80.2% and 84.0%, respectively).

Kasimanickam *et al*., (2021) studied pregnancy rate per AI (PR/AI) and breeding season pregnancy rates between insemination with sexed semen (SS; at 18 hr after the onset of oestrus) and conventional semen (CS; at 12 hr after the onset of oestrus,) and offspring gender ratio between two groups were compared. Angus cross cows (n = 686, during 2019 and 2020 breeding seasons) were oestrus-synchronized using Select-Synch + CIDR protocol and were observed thrice daily for oestrus until 72 hr after PGF2α administration. Cows expressed oestrus (n = 513) were inseminated with either SS (n = 246; SexedULTRA 4M™; y chromosome-bearing sperm) or CS (n = 267). Cows (n = 173) that failed to express oestrus at 72 hr after PGF2α received 100 µg of GnRH and CS insemination concomitantly. Cows that expressed oestrus, PR/AI did not differ ($p > .1$) between SS (65.0%) and CS (66.7%) groups. The overall PR/AI differed ($p < .05$) between SS (65.0%) and CS (56.4%) groups. The natural service PR differed ($p < .001$) but breeding season PR ($p > .05$) did not differ between SS vs. CS groups.

2.2.2 Fertility response in estrus synchronized pluriparopus cows with conventional semen.

Bagal and Kadu, (1989) observed administration of 5 ml GnRH in postpartum crossbred cows as 66.66% came into estrus and recorded as 100% conceived within 81 days requiring 1.16 services per conception.

Twagiramungu *et al*., (1992) observed postpartum beef cows and heifers in Group I received 8µg of buserelin on Day 0 (the beginning of the experiment) and 500µg of cloprostenol (PGF) on Day 6 (GnRH I, n = 54). In Group 2 (GnRH II, n =54). The females were injected with buserelin on Day 0 (8µg) and Day 3 (4µg), and PGF on Day 6 and Day 9. Between Days 6 and 12, the overall synchronization rate (85.2 vs. 92.6%), pregnancy rate (57.4 vs. 68.5%) and conception rate (67.4 vs. 74.0%) were similar for both treatment groups.

Macmillan and Peterson, (1993) synchronized 724 cows with CIDR with or without PGF2α inserting the CIDR for 7, 14, and 21 days in group I, II and III, respectively. They reported percentage insemination at 48 hrs after removal of 8 CIDR to be 52.6%, 74.5% and 74.7%, respectively and calving rate to be 57.7%, 44.4% and 39.7%, respectively for CIDR inserted for 7, 14 and 21 days.

Wolfenson *et al.,* (1994) studied on follicular development between estrus synchronized and untreated cows and reported that the use of Buserelin (8µg) on day 12 and 7 days before synchronization of estrus with PGF2α altered follicular development and produced preovulatory follicles which were more homogenous, more estrogen-active and more dominant with a greater size difference between the preovulatory and subordinate follicles prior to estrus.

Pursley *et al.,* (1995) reported a new synchronization method for synchronizing the time of ovulation in cattle using GnRH and PGF2α. In Experiments 1 and 2, a total of 20 lactating dairy cows ranging from 36 to 280 d postpartum and 24 dairy heifers were treated with an intramuscular injection of 100 µg GnRH at a random stage of the estrous cycle. 7 d later the cattle received PGF2α to regress corpora lutea (CL). Heifers and Lactating cows received a second injection of 100 µg GnRH 24 and 48 h later, respectively. After 24 h of the second GnRH injection, lactating cows were artificially inseminated. Lactating cows received the injection of PGF2α 48 (Group l), 24 (Group 2), and 0 h (Group 3). In Experiments 1 and 2, the first injection of GnRH caused ovulation and formation of a new or accessory CL in 18/20 cows and 13/24 heifers. after PGF2α, CL was regressed in 20/20 cows and in 18/24 heifers: Ten of 20 cows conceived to the timed AI. In Experiment 3, the conception rate in Groups 1 and 2 was greater than in Group 3, (55 and 46 % vs. 1 l %, respectively).

Stevenson *et al*., (1996) reported the effect on fertility of GnRH when used in conjunction with one or two injections of PGF2α. Conception was reduced from 63.5% for 74 controls to 48.7% for the 79 heifers and cows that had been treated with GnRH, but estrus detection and pregnancy rates were similar. Although conception rate was not different, one fixed-time insemination after the GnRH, PGF2α and

GnRH treatment tended (35.3%) to reduce fertility compared with effects of the control (47.1%).

Pursley *et al*., (1997) performed a method of timed AI using GnRH and PGF2α in which ovulation was synchronized within an 8-h period. Ovulation was synchronized by a second injection of GnRH given 2 d after PGF2α. In 20 cows, ovulation was synchronized within an 8-h period, 24 to 32 h after the second injection of GnRH. This synchronized ovulation was possible because pre ovulatory follicles were at a similar stage of growth and were responsive to LH at the time of the second GnRH injection. This precise synchrony of ovulation resulted in similar pregnancy rates per AI after timed AI compared with cows bred to a detected estrus.

Roy and Twagiramungu (1997) observed 61% conception rates during induced estrus in cows treated with GnRH–PGF2α–GnRH protocol.

Geary *et al*., (1998) reported efficacy of Ovsynch protocol on pregnancy rates of beef cows. Pregnancy rates were higher ($P<0.025$) for Ovsynch-treated cows (54%). Pregnancy rates of cyclic Ovsynch- treated cows (59%). It was concluded that the Ovsynch protocol is capable of inducing a fertile ovulation in cyclic and anestrous beef cows and that pregnancy rates to a timed insemination.

Fricke *et al*., (1998) recorded ovulation of a dominant follicle in response to the second GnRH injection in 85% of lactating cows receiving ovsynch protocol.

Pursley *et al.*, (1998) asserted that conception rate in cows was reduced if AI was performed at 32 h after the second GnRH treatment in the Ovsynch protocol or just after ovulation compared with AI performed at 18-24 h after detected estrus. They found 39 % conception rate in animals after treatment. PR/AI using conventional semen was greater ($P < 0.05$) for second parity as 48 % as compared to first parity (37 %) and third parity (35 %).

Dantre and Kumar, (1999) treated with Receptal yielded 85.71% heifers expressing estrus within 23.5±1.89 days post treatment with 66.66% conception rate.

Keister *et al*., (1999) reported 70% pregnancy rate in cows following Ovsynch protocol.

Thompson *et al*., (1999) reported in cattle synchronization with Ovsynch is associated with pregnancy rates of between 30-40% although pregnancy rates of 20 % have also been reported.

Whisnant *et al*., (1999) observed synchronization protocols and found 34.9 and 34.9%, conception and pregnancy rates respectively in dairy cattle. Conception and pregnancy rates from breeding at detected estrus after PGF2α averaged 37.7 and 19.8%, respectively.

Stevenson *et al.,* (1999) worked on 1308 Holstein cows synchronized using ovsynch protocol. GnRH injection was administered on initial day followed by PGF2α injection on day 7, then another GnRH injection 33 h later, and AI 16 to 18 h after the second GnRH injection. They found conception rate as 22.1 % in animals.

Vasconcelos *et al.,* (1999) reported that the overall synchronization rate in cows using Ovsynch protocol was 85 % and there were clear differences in response according to the day of protocol initiation. They have also recorded that 64 % of cows ovulated to the first GnRH and 93 % of the cows showed low progesterone at second GnRH after PGF2α administration. They found overall pregnancy rate using conventional semen during AI as 61 % in animals after treatment.

Sendag *et al*., (2001) synchronized anestrus and subestrus dairy cattle and heifer using a progesterone releasing intravaginal device (PRID). PRID was administered for 10 days to 8 heifers and 12 cows. A single injection of 500µg cloprostenol, PGF2α analogue, was given on the day of PRID removal. The animals were inseminated 48, 72 and 92h after PRID removal. The pregnancy rate of anestrus and subestrus cows were 81.8% and 62.5% respectively. The overall onset of estrus and pregnancy rate in animals with PRID application was 95% and 73.6% respectively. PRID was effective for the treatment of animals with anestrus and subestrus conditions.

Lucy *et al.,* (2001) conducted experiment involving postpartum beef cows to compare the effectiveness of CIDR protocol to a single PGF2α injection and compared with non-synchronized (control) females. It was recorded as a greater incidence of estrus during the first 3 day of the breeding period in CIDR+ PGF2α -

treated cows compared with PGF2α treated or control cows (15, 33, and 59% for control, PGF2α and CIDR+ PGF2α, respectively; P<0.001). They observed as improvement in pregnancy rate for the 31-day breeding period for cows treated 9 with CIDR+ PGF2α (50, 55, and 58% for control, PGF2α, and CIDR+ PGF2α, respectively, P=0.10).

Barufi *et al.*, (2002) observed estrus in Nellore cows using Crestar or CIDR (new or used intra-vaginal progesterone releasing implants) in combination with the administration of 500IU of eCG at implant removal. There was no significant difference in the pregnancy rate of cows given the new or used CIDR (28.8 vs. 38.7%). Highest pregnancy rate in Crestar (52.7%) compared to the two CIDR systems.

Martinez *et al.*, (2002) observed the pregnancy rate at induced estrus was 42.9% in cows treated with CIDR protocol.

Kesler, (2002) treated anestrus beef cows with CIDR and recorded as higher pregnancy rate (26%) and conception rate (57%) than control group (38% and 4%), respectively while cyclic cows showed higher (11%) pregnancy rate with conception rate (58%).

Kim *et al.*, (2003) evaluated pregnancy rates in lactating Holstein cows treated with an Ovsynch protocol (GnRH–PGF2α–GnRH) to determine the factors that may influence pregnancy rates following protocol treatment. In their experiment lactating Holstein cows n=34) were assigned to an injection of GnRH (d 0), an injection PGF2α (d 7), second injection of GnRH (d 9) and TAI using conventional semen 16 h after the second GnRH injection. The Pregnancy rate after TAI following this protocol was observed as 20.6 %.

Peters and Pursley (2003) reported that synchronization of ovulation (ovsynch) in lactating dairy cows was an efficient method for controlling the time of first and subsequent AI. They conducted experiment to determine, the effect of administering the final dose of GnRH on the same day as prostaglandin F2a (PGF2a) administration. Lactating dairy cows (n=218) were randomly assigned to receive either Ovsynch or the modified version of Ovsynch (MOV; cows were given 100 mg

GnRH, then 7 days later cows were administered 25 mg PGF2a followed immediately with 100 mg GnRH). In both treatment groups, AI took place 16 h after the final administration of GnRH. Pregnancy rate /AI (PR/AI) was greater ($P < 0:025$) in OV (31.3 %) versus MOV (14.7 %).

Tenhagen *et al.,* (2003) synchronized 136 Holstein Frisian cows using Buserelin (GnRH analogue) at day-10, Tiaprost (PGF2α analogue) at day -3 and again GnRH at day -1. Timed artificial insemination (TAI) was carried out 16–20 h after the second dose of GnRH on day 0. The overall synchronization rate (proportion of cows with ovulation within40 h after GnRH) was 86.8 % in primiparous and 88.2 % in multiparous cows, respectively Conception rates were numerically higher in primiparous than in multiparous cows (39.7 % vs. 29.4 %, $p > 0.05$).

Dejarnette and Marshall (2003) opined that though ovsynch allowed acceptable pregnancy rates in cows with no heat detection, it does not eliminate the need for heat detection. He also stated that natural heats can occur on any given day. As many as 20 % of cows will display standing estrus between days 6 and 9 of the Ovsynch protocol and conception rates in these animals will be compromised if bred strictly on a timed AI basis. They found pregnancy rates at TAI in cows receiving post synchronization treatments (Ovsynch = 29 %, 50/175 versus Co synch = 22 %, 38/173).

Lamb *et al.,* (2004) studied CIDR + PGF2α treatment improved the synchrony of estrus compared with the PGF2α alone, with 60% vs. 25%, of heifers in estrus over 3 d after CIDR inserts were removed.

Chenault *et al*., (2003) reported much lower values with non-significant difference in conception rate and pregnancy rate of lactating HF cows for CIDR-treated (26.7%, 12.2%) and control (30.9%, 11.1%) groups and concluded that CIDR inserts improved synchrony of returns to estrus, slightly reduced pregnancy rate to initial AI, but did not affect conception rate or pregnancy rate to AI during the resynchrony period.

Baruselli *et al*., (2004) observed the pregnancy rate at induced estrus was 52% in cows treated with CIDR protocol.

Colazo *et al*., (2004) observed the pregnancy rates following fixed-time AI (FTAI) in beef cattle given a new or previously used CIDR insert and injections of estradiol, with or without progesterone, to synchronize follicular wave emergence. They reported pregnancy rate in heifers as 46.9% and 49.6% treated with new CIDR plus an intramuscular injection of 1mg estradiol cypionate with or without 100mg of a commercial progesterone preparation at the time of CIDR insertion. The study was concluded as pregnancy rate to FTAI did not differ between cattle synchronized with a new or once-used CIDR.

El-Zarkouny *et al*., (2004) used CIDR with Ovsynch and reported pregnancy rates at 29 days (59.3% vs. 36.3%) and 57 days (45.1% vs. 19.8%) with Ovsynch + CIDR vs. Ovsynch alone.

Naidu, (2004) observed number of services per conception as 1.70 ±0.18 in postpartum anestrus cows in GnRH –PGF2α–GnRH protocol.

Tenhagen *et al*., (2004) studied the differences between primiparous (583) and Pluriparous (1001) cows after synchronization of ovulation with an Ovsynch protocol in 6 trials. Synchronized with an Ovsynch protocol consisting of a GnRH at days 0 and 9 and PGF2α analogue on day 7, AI was carried out in all cows 16–20 h after the last treatment. Conception rates to TAI were higher in primiparous than in older cows (37.9 vs. 31.6%, P=0.015). Pregnancy rates were higher in primiparous cows. They concluded that Ovsynch protocol is more effective in primiparous than in older cows.

Bartolome *et al.*, (2005) reported Pregnancy rates on days 27, 45 and 90 were 25.2%, 17.5% and 13.9% for Ovsynch protocol

Keith *et al*., (2005) reported 52% pregnancy rate in cows after FTAI following Ovsynch protocol.

Portaluppi and Stevenson (2005) determined pregnancy rates in dairy cows (n=211) using ovsynch protocol receiving injections of GnRH 7 d before and 48 h after the PGF2α injection. Timed AI (TAI) was performed at the time of the second GnRH injection or 24 h later. They recorded conception rate as 23.5 %.

Rabiee *et al.*, (2005) investigated the efficacy of the ovsynch program in lactating dairy cows to improve conception and pregnancy rates as compared with untreated controls and other synchrony programs. The synchrony program included Gonadotropin-releasing hormone (GnRH) injection followed by an injection of PGF2*α* 7 d later, and a second GnRH injection given 48 h after PGF2*α*.

Bello *et al.*, (2006) observed that the ovulatory response in cows after the first GnRH administration was a critical factor for successful synchronization of ovulation in ovsynch protocol and also stated that high ovulation rate after the first GnRH treatment increased the likelihood that cows will have a functional dominant follicle capable of ovulation during the final GnRH treatment.

Crane *et al.*, (2006) studied the effectiveness of the Ovsynch protocol under commercial conditions for the treatment of cystic ovarian disease in dairy cattle. A total of 401 lactating dairy cows with ovarian cysts were alternatively allocated to two treatment groups on the day of diagnosis. Cows in the Ovsynch group were treated with GnRH on Day 0, PGF2α on Day 7, GnRH on Day 9, with timed insemination 16–20 h later. The percentage of cows with a CL on day 21 for the Ovsynch groups was 83%, respectively ($P>0.05$). Conception and pregnancy rates for cows in the Ovsynch group were 18.3 and 14.4%, respectively, concluded that fertility was not different between cows with ovarian cysts treated with either the Ovsynch or the CIDR protocols in this dairy herd.

Stevenson *et al*., (2006) carried out the treatment of cycling and non-cycling lactating dairy cows with progesterone during Ovsynch. Overall conception rates at 28 day (40% vs. 50%) and 56 day (33% vs. 38%) after TAI differed between OVS and OVS + CIDR, respectively. When treatment response x location interaction was studied, it was found that pregnancy outcomes were more positive for OVS + CIDR.

Kasthuri, (2006) recorded conception rate in GnRH–PGF2α–GnRH treated postpartum anestrous crossbred cows as 40.00 per cent in first service conception and over all conception rate as 70.00%.

Singh *et al.,* (2006) reported the estrus induction and fertility response after CIDR and eCG treatment in 30 true acyclic Sahiwal cows (15 cows, ≥ 90 days postpartum; 15 post pubertal heifers, ≥ 30 months of age) and a similar 20 untreated controls (10 cows, 10 heifers) and found as 100% estrus induction response in treated cows and heifers within 24-36 and 36-48h, respectively after CIDR withdrawal; with mean intervals of 44±3.18 and 48±2.35h, respectively. The conception rate at induced estrus was higher in cows (40%) than heifers (20%) and pregnancy rates after 2 subsequent estruses were 80 and 60% in cows and heifers, respectively (overall 70% for all treated animals). In comparison, only 10% of control animals (2 cows, 2/20) showed estrus and became pregnant (10%).

Wheaton and Lamb, (2007) studied the percentages of cows initiating estrous cycles in the CIDR+ PGF2α groups were 55% treatment initiated approximately 40 day postpartum synchronized estrus cyclicity in 76% of primiparous and 43% of multiparous beef cows. Treatment with CIDR–PGF2α provides an effective reproductive management tool to increase the proportion of cows initiating estrous cycles by the start of the breeding season.

Mohammedsadegh *et al*., (2007) reported the services per conception in anestrus cows treated with Ovsynch as 1.94±0.2.

Ansari, (2007) recorded 60% first service conception rate and overall conception rate was found to be 90% in GnRH–PGF2α–GnRH treated cows.

Ali and Fahmy, (2007) observed the ovarian dynamics and progesterone concentrations in cyclic and non-cyclic buffalo-cows during Ovsynch program and observed lower conception rate in non-cyclic cows (37.5%) than cyclic cows (60%).

Dagli *et al*., (2009) studied 6 HF crossbred cows at average 180 days postpartum period with injection Receptal 5ml intramuscular on day 1^{st}, injection Lutalyse 5ml intramuscular on day 7^{th} and repeat dose of inj. Receptal on day 9^{th} with fix timed insemination on day 11^{th} morning. The establishment of cyclicity in 100.0% and conception rate in 33.0% cows with Ovsynch protocol.

Ozyurtlu *et al*., (2009) found pregnancy rates and overall conception rate were 44, 53.85% and 73.08, 53.85% with Norgestomet and PRID protocol in acyclic HF heifers respectively.

Muneer *et al*., (2009) reported the number of services required per conception in anestrus postpartum crossbred cows treated with Ovsynch and control groups as 1.44 and 1.66 respectively.

Ravikumar *et al*., (2009) obtained ovulatory response and first service conception rates in buffaloes were 83.33% and 33.33% with Ovsynch and 100.00% and 41.66% with Ovsynch plus CIDR groups, respectively.

Ghallab *et al*., (2009) synchronized ovulation in 40 HF dairy cows which divided into 4 group according to lactation number (1^{st} n=13; 2^{nd} n=7; 3^{rd} n=14 and 4^{th} n=6 lactation) during the summer and treated with Ovsynch protocol for TAI. Highest pregnancy rate was found in 4^{th} parity (50.00 %), followed by 2^{nd} and 3^{rd} parity (42.85%) and lowest in 1^{st} parity (30.70 %), with an overall pregnancy rate of 40.00%.

Takahashi *et al*., (2009) observed the synchronization and pregnancy rate in dairy cows treated with Ovsynch protocol with two different intervals between the PGF2α and 2^{nd} GnRH injections. A total of 27 Holstein cows were injected with a GnRH analogue on day 0 and a PGF2α analogue on day 7. A 2^{nd} GnRH injection was administered 30 hr after the PG injection to 13 animals (30-hr group), and 48hr after the PG injection to 14 animals (48-hr group). Timed AI was performed 16hr after the 2nd GnRH. Synchronization rate and pregnancy rate were respectively 84.6 and 46.2% in the 30-hr group and 85.7 and 50.0% in the 48-hr group, with no significant difference between the two groups.

Polat *et al*., (2009) observed progesterone supplementation with a progesterone releasing intravaginal device (PRID) for 12 days in heifers that had delayed onset of puberty resulted in 93.9% of the heifers being cyclic (P4 $\geq$ 1ng/ml) within 72 hours of PRID removal. Fixed-time artificial insemination at 48 and 72 hours after withdrawing the PRID produced 54.6% combined pregnancy rate at 60 days post-insemination.

Cevik *et al*., (2010) reported on comparison of pregnancy rates between Heatsynch and CIDR-based synchronization protocol in dairy cows and observed that the pregnancy rates after first AI for Heatsynch and CIDR-based groups as 80.00%, and 53.33%, respectively. In second AI pregnancy rates of Heatsynch, and CIDR-based groups were found as 100%, and 71.43%, respectively. comparison of pregnancy rates after timed artificial insemination in Ovsynch protocol in dairy cows and pregnancy rates after first AI for Ovsynch groups were observed as 76.92%. In the case of the second AI pregnancy rates of Ovsynch group were 100%.

Sa Filho *et al*., (2010) observed pregnancy rate of 51.4% by using progesterone based estrus synchronization protocol in Bos indicus cows.

Deshmukh *et al*., (2010) reported Ovsynch protocol in 36 postpartum Red Kandhari cows resulted as 50.00% conception/pregnancy rate.

Mohan *et al*., (2010) observed the Ovsynch protocol in postpartum anestrus Sahiwal cows (n=10), which had not shown any sign of estrus even after four months of calving and pubertal anestrus Sahiwal heifers (n=10) aged>3 years (3-3.5 years) and weighing 250-350kg, and a group of anestrus Sahiwal cattle (n=10) was kept as control. All the cows and heifers were inseminated at 12 and 24h after the second GnRH injection without observing heat (FTAI). Pregnancy diagnosis was performed in all the experimental animals after 30days of AI. The conception rate was recorded as 10 per cent in the control, while 30 (cows) and 20 (heifers) % in the experimental groups.

Keskin *et al*., (2010) reported Ovsynch protocol in lactating dairy cows began with GnRH (10μg) in the GPG group (GnRH-7d-PGF2α-56h-GnRH-18h-AI), The conception rate was found to be 48.4%.

Whitley, (2011) observed 26.9±2.5% pregnancy rate in cows after receiving Presynch–Ovsynch.

Ghuman *et al*., (2011) studied 18 lactating repeat breeder cows with ovsynch plus CIDR protocol animals were subjected to fixed AI at 24h after second GnRH (Day 10) without estrus detection. Sensitivity of the plasma progesterone solid phase RIA assay was 0.1ng/ml; intra-assay and inter-assay variation coefficients were 6.5% and 9.0%, respectively.

Islam, (2011) reported the synchronization of estrus in cattle and concluded that the GnRH may be helpful to synchronize the estrous cycle in delayed pubertal heifers and postpartum anestrus cows and further a single timed artificial insemination is possible with this method.

Ergene, (2012) reported the effects of two protocols on fertility parameters in fifty post-partum anestrus cows received Ovsynch protocol. The percentage of estrus detection as 68%. The pregnancy rates after artificial insemination as 47.05% ($P<0.05$).

Ramakrishnan and Dhami (2012) synchronized postpartum anestrus/subestrus Gir cows (n=18) which includes anestrus/subestrus 6 cows and 6 normal cyclic cows were kept as control. Three standard synchronization protocols initiated around day 90 to 92 postpartum were Ovsynch considering the day of first GnRH injection or CIDR insertion as day 0. The animals were bred by fixed-time Al (FTAI). Pregnancy was confirmed per rectum on day 45 post-AI. The conception rates at induced estrus (FTAI) in Ovsynch protocol were 50.00%. The corresponding overall conception rates of 2 cycles post-treatment were 66.66 (4/6) %. In control group, the first service and overall conception rates were 33.33 and 66.66 (4/6) %, respectively.

Baruselli *et al.,* (2012) asserted that timed artificial insemination (TAI) can be applied routinely in the reproductive programs on farms. TAI protocols are designed to promote control of both luteal and follicular functions, permitting the TAI with satisfactory pregnancy rates per AI. Most common of TAI therapies use GnRH or estradiol plus progesterone/progestin (P4)-releasing devices and prostaglandin F2α. Moreover, TAI programs should be considered as an important tool for reproductive management to enhance the reproductive performance of cattle.

Caraba and Velicevici, (2013) used Ovsynch Protocol versus Cosynch Protocol in Dairy Cows. They studied on 15 dairy cows which were synchronized by Ovsynch and Co synch programs. The estrus response for cows in Ovsynch protocol was of 63%. Pregnancy per insemination at 60 days was of 25%. Estrus response for cow in Cosynch protocol was of 57%. Pregnancy per insemination at 60 days was of 57%. Synchronization of ovulation using Ovsynch protocols can provide an effective way to manage reproduction in lactating dairy cows.

Colazo and Mapletoft (2014) presented a review of the physiology and endocrinology of the estrous cycle and explained how ovarian physiology can be manipulated and controlled for timed artificial insemination (TAI) in beef and dairy cattle. TAI programs have become an integral part of reproductive management in many dairy herds and offer beef producers the opportunity to incorporate AI into their herds. Gonadotropin-releasing hormone-based protocols are commonly used for estrus synchronization as part of a TAI program. Protocols that increase pregnancy rates in lactating dairy cows and suckling beef cows have been developed.

Dhami *et al.*, (2015) mentioned use of ovsynch protocol recorded as 100% estrus induction with conception rates at induced estrus of 50% the overall of three cycles as 80%.

Barolia *et al*., (2016) studied comparative study of co synch and ov synch protocol on fertility in repeat breeder gir cow Present study was done on organized college farm. A total of 18 repeat breeder cows were randomly divided in to Co synch, Ovsynch and control group. All the repeat breeder Gir cows from Co synch and Ovsynch protocol exhibited estrus signs (100%) within 48-72 hours after PGF2 alpha injection. Pregnancy rate recorded for Co synch, Ovsynch and control group were 50%, 66.66%, 33.33% respectively. In conclusion, this study indicates that, Ovsynch protocol is more efficacious in settling pregnancy than Co synch protocol in repeat breeder Gir cows (66.66% vs.50%).

Jeong *et al*., (2016) observed the effects of Gonadotrophin-releasing hormone (GnRH) administration and a controlled internal drug-releasing (CIDR) insert after timed artificial insemination (TAI) on the pregnancy rates of dairy cows. The probability of pregnancy following TAI did not differ between the GnRH (34.4%) and control (31.6%, $P > 0.05$) groups. the probability of pregnancy following TAI was higher (odds ratio: 1.74, $P < 0.05$) in the CIDR group (51.1%) than in the control group (39.3%).

Karaca *et al*., (2016) evaluated the efficacy of reducing GnRH dose on the ovulation and conception rate, and size of the ovarian structures following the Ovsynch program in lactating cows. The cows were allocated randomly into two treatment groups (full dose; FD, n=20 and half-dose; HD, n=20). Cows in the FD group were treated with 10.5µg buserelin acetate on day 0, with 0.150 mg D-cloprostenol 7 d later and with 10.5 µg buserelin acetate 2 d later. Estrous cycles in the HD group were synchronized using the same scheme as FD-treated cows, but the dose of buserelin acetate was reduced to5.25 µg at both GnRH administration times. Cows were inseminated with conventional semen at the 16-20 h after the second GnRH administration. No significant differences were observed in the dominant or ovulatory follicle diameters in FD and HD groups. The conception percentages did not differ statistically between the HD (50 %) and FD (40 %) groups.

Nowicki *et al*., (2017) described that the ovsynch program in dairy management enables artificial insemination (AI) to be performed at the precise optimum time without control of the ovaries and uterus. Use of such protocols in reproductive management allows estrus cycles to be synchronized and cows to be effectively inseminated without estrous detection, which is time-consuming and difficult in farms with numerous cows. Therefore, ovsynch has become the first management tool for AI and is an alternative method to heat detection. Over the 20 years since its first implementation, ovsynch has been modified many times to improve its reproduction outcomes and widen its use.

Jaskowski *et al*., (2018) analyzed the efficacy of the ovsynch protocol is determined by various individual and environmental components such as body condition, age, ovarian status, co-existing health problems, season, outdoor temperature, year, time of the ovsynch onset and to a minor extent production level. The efficiency of the ovsynch program can be increased by introducing modifications to it and by making adjustments regarding its starting date after calving. Interactions of the ovsynch and its modifications with the season and age of cows (primiparous, multiparous) and their relationship with fertility seem to indicate the need for a precise individual selection of the synchronization protocol taking into account the influence of those factors.

Khalil and Hussein (2019) reported the conception rate by insemination at standing heat results in a similar or higher pregnancy rate compared with FTAI. In this study, a total of 8944 inseminations were included. All cows were subjected to Pre synch ovsynch protocol. Cows detected in estrus (n=7424) were artificially inseminated, whereas cows not observed estrus were submitted to FTAI. They observed conception rate was 36.64 ±1.32 using FTAI.

2.3 Estrual discharge Properties at Estrus and Conception Rates in Cows

Sukhdeo and Roy (1971) investigated the fern pattern in cows related to the typical, atypical and zero fern grade and It was found that in buffaloes 75.00 per cent those with typical, 37.00 per cent with atypical and 05.00 per cent in zero fern pattern become pregnant.

Adhalikar and Dubey (1986) reported that the percentage of typical fern pattern in ovulatory and unovulatory heat was 75.50 and 24.50 per cent, respectively.

Das *et al.,* (1988) observed conception rate in typical, atypical and nil fern pattern as 75.00, 25.00 and zero per cent, respectively in cows and claimed more chances of ovulation in cows showing typical fern pattern.

Nair and Kharche (1989) observed that continuous fern pattern was higher in repeat breeder than normal cows and normal cyclic cows had a clear defined fern pattern.

Salphale (1993) observed that in normal cows after synchronization the number of cows showing typical fern pattern reduced from seven to five where as in repeat breeders it increased from three to five.

Jadhav (1996) observed fern pattern of cervical mucus as typical, atypical and nil in 63.7, 34.28 and 0.0 per cent for fertile estrus while 69.3, 23.1 and 7.7 per cent for non fertile estrus, respectively.

Srivastava *et al.*, (2000) observed cervical mucus from 60 cross bred cows at AI and found that 55.26 per cent cows with typical fern pattern and only 18.77 per cent of cows with atypical fern pattern become pregnant but 25.97 per cent cows with nil fern pattern could not conceive.

Tsiligianni *et al*., (2000) determined the physical properties of cervical mucus of cows exhibiting spontaneous estrus in relation to fertility. A study was executed on 93 cows of the Friesian breed. The animals exhibited spontaneous estrus, without being submitted to any hormonal treatment. Samples of cervical mucus were collected 5-30 min before AI and properties like pH, crystallization 6 and viscosity rate of cervical mucus were analyzed. The results obtained from pregnant cows (n=44) were compared to those obtained from non pregnant cows (n=49). a) Viscosity had been significantly lower ($p < 0.05$), b) crystallization had been significantly higher ($p < 0.05$), and c) pH of cervical mucus did not differ. In conclusion, the best time for AI was when viscosity was below 20 mm H2O and crystallization was above 3. Viscosity and crystallization could be related to ovulation time, but this needs further investigation.

Kumaresan *et al.*, (2001) reported the conception rate as 63.63 and 21.42 per cent in cattle inseminated when cervical mucus showed typical and atypical fern pattern, respectively.

Rangnekar *et al.,* (2002) reported higher frequency of fern pattern in estrus stage and conception rate was highest when there was typical fern pattern whereas the same was zero in nil fern.

Bennur *et al*., (2004) designed an experiment to evaluate a relationship between the physio-chemical properties of the cervicovaginal mucus of cows. The cervical mucus was collected aseptically prior to AI from 44 cycling pluriparous cows and mucus samples were subjected for the study of arborization and pH. The percentage of typical and atypical arborization patterns of CVM was 87.50 % and 12.50 % in pregnant and 80.00 % and 20.00 % in non-pregnant cows, respectively.

The mean pH of CVM of pregnant and non-pregnant cows was 8.13 and 8.15, respectively.

Predojevic *et al*., (2007) studied the physical property of arborization on sperm penetration and conception rate in cows. The results of their investigation showed that the mucus with typical ferning had a better sperm penetration and conception rate of 63.53 % than ($p<0.05$) the mucus with atypical crystallization pattern with conception rate of 41.17 %.

Nayak *et al.*, (2009) reported better conception rate when animals were bred with good to satisfactory arborization pattern and none of the animals with missing arborization pattern was found to be conceived. Typical fern pattern indicative of quality cervical mucus is related with higher chances of conception and in repeat breeder crossbred cows confirmation of fern pattern is more appropriate to adjudge the insemination attempt.

Dodamani *et al*., (2010) examined twenty-four Deoni repeat breeder cows randomly allocated into 4 groups I, II, III and IV of six each. The animals of groups I, II and III were administered with injection of 250 μg of buserelin acetate (Receptal®) on two occasions. Among the physical characters of estrual cervico-vaginal mucous, they observed that a typical arborization pattern of 80.95 % in pregnant vs. 55.56 % in non-pregnant cows favored better fertility, although the differences between the groups were statistically insignificant. However, the pH of estrual cervicovaginal mucous did not indicate any effect on fertility and it ranged within 8.00 to 9.00.

Lim *et al*., (2014) correlated the physical characteristics of estrus mucus and conception rates in dairy cattle. Samples of estrus mucus from the cervix were collected from 108 dairy cattle during the heat and were examined for consistency. Samples were taken from animals starting from day of breeding to the completion of one estrus cycle. The consistency of the cervical mucus was based on the thinness and thickness of the cervical mucus. The cows were bred and the pregnancy diagnosis was performed at the 60th day post-breeding. Findings showed that the mucus consistency of cervical mucus in the dairy cattle was thin in 74.1 % and thick in 25.9 % animals. The conception rates of dairy cattle with thin and thick consistency of cervical mucus were 81.8 % and 18.2 %, respectively. Pregnancy was associated with the consistency of cervical mucus ($p<0.05$). Findings indicated that dairy cattle with thin consistency of cervical mucus having remained pregnant.

Bernardi *et al*., (2016) executed a study to characterize the cervical mucus (CM) collected during insemination in Holstein cows and to relate the secretion pattern with pregnancy. The mucus was collected from mid-cervix from 64 cows with spontaneous estrus (SE) and induced estrus (IE). The quantity (copious, moderate, absent), appearance (clear, cloudy and dirty), consistency (thick, medium, watery), arborization and pH of the mucus were observed. Mucus secretion of cows with SE was significantly different from those with IE, showing a lower degree of crystallization. Pregnancy was associated with a copious, clear and watery discharge (similar to egg white), with an arborization degree of 2.25, presence of atypical fern leaves together with rosette formations and needles or thorns on rails due to low levels of progesterone accompanied by high concentrations of estrogens.

Parikh *et al*., (2018) reported the study on association of estrus behaviour and cervical mucus properties with conception in postpartum Gir cows and observed that the 46.81% estruses had arborisation pattern either typical or atypical. Estrus cows with clear appearance of mucus and typical arborisation pattern had higher conception rate.

Ningwal *et al*., (2018) conducted study on 20 crossbred cows and 20 heifers and collected Cervico-vaginal mucus samples (CVM) at estrus before AI and were immediately evaluated for physical properties particularly fern pattern. After 2 months of insemination, pregnancy in cows was confirmed by rectal palpation. The per cent incidence of typical fern pattern of CVM in conceived crossbred cows was recorded as 86.67 % whereas in heifers it was 83.33 %. The frequency of atypical fern pattern of CVM in conceived cows was 13.33 % and in 8 heifers was 16.66 %.

Physical profile revealed that the variations in per cent incidence of typical, atypical and nil fern patterns of CVM samples were highly significant (P<0.01) in both the groups of conceived and non-conceived cows and heifers. On the basis of results they concluded that the typical fern pattern of CVM favors conception in cattle.

Damarany (2020) analyzed conception rate of Egyptian Baladi cows as influenced by vaginal mucus discharge (VMD). Total 40 cows were used in the experiment and were followed up after two-week post-partum. Vaginal mucus discharges were divided into different categories according to viscosity (thin and thick) or pH (7-7.5, 7.5-8 and >8). The conception rate was 87.5 % (n=32) in cows which had thin vaginal mucus during estrus which was significantly higher as compared to pregnancy rate of 25 % in those cows (n=8) which had thick vaginal mucus. The conception rate was significantly higher (78.6%) in cows which had vaginal mucus pH level at estrus >8 compared with those cows which had vaginal mucus pH value ranging from 7-7.5 (25 %) to 7.5-8 (62.5 %). The results clarified that the cows which had vaginal mucus discharge (VMD) thin, and pH value at estrus >8 revealed higher conception rate.

2.4 Estrus detection by use of electronic heat detector

Several electronic devices have been developed to alleviate the need for visual estrus detection in cows and to provide a precise determination of the onset of estrus. Commercially available electronic technologies for estrus detection are based on changes in physical activity (pedometers), changes in electrical and chemical properties of the reproductive tract (intravaginal resistance probes), or mounting activity (mount detectors) (Rorie *et al.*, 2002). The vaginal electrical resistance fluctuates with stage of the estrous cycle, is highest during the luteal phase, and declines during the follicular phase. The lowest VER values are correlated with the preovulatory LH peak (Schams *et al*, 1977, Canfield and Butler, 1989).

Ivanov, (1964) reported that electrical resistance of the vaginal mucus in cows varied between 150 and 400 ohms. It was 150-200 ohms at the onset of estrus and this reading was related to high conception rate.

Aizinbudas and Norvaishas (1966) observed the least electrical resistance (200-250 ohms) 12-15 h after the onset of estrus. The cows accepted male only when resistance was 200-400 ohms and standing reflex was greatest sometime after resistance between these limits was first noted.

Cerne, (1968) noticed that the electrical resistance was lower during estrus than at the other phases of estrus cycle, it increased slightly at the height of estrus when the standing reflex secured. When tested in 311 cows, electrical resistance was 65-150 ohms, during estrus and 285-440 ohms during anestrus, respectively

Aizinbudas *et al*., (1969) have shown that the electrical resistance of cervical mucus for 23 cows in control was 435 ohms in diestrus and 261 ohms in estrus as against 547 and 352 ohms, respectively for 25 cows given a diet deficient in Vitamin A.

Stan, (1969) recorded that electrical resistance of the vaginal secretion of cows was at least 700-750 ohms at diestrus and it decreased to the level of 150-200 ohms, 10-20 h after the beginning of heat.

Turtion, (1969) in a review on electrical resistance of vaginal mucus reported that the difference in the electrical resistance during different stages of estrus cycle is due to changes occuring in the vascularity and secretary status of the mucosa.

Vorobjev and Grenaderskii (1969) observed that the electrical resistance of the vaginal mucosa of cows was lowest (200-270 ohms) 12 h before ovulation and coincided with the best time for insemination.

Edward and Levin (1974) described the method of proper heat detection based on measurement of changes in electrical resistance of mucosal surface of vestibular epithelium. They reported an average conception rate of 75.00% by using this method for heat detection.

Schiavo *et al*., (1975) observed that mean electrical resistance of vaginal mucus was 44 ohms for cycling and cystic Holstein cows. They concluded that the electrical resistance was not reliable for distinguishing different physiological status.

Kopytin, (1976) studied the electrical resistance of vestibular mucosa for 61 and 28 Hareford cows. It was 370-800 and 373-513 ohms during proestrus and 289-345 and 500-446 ohms during estrus, respectively.

Gartland *et al*., (1976) analyzed the correlation of electrical resistance of vaginal mucus and milk progesterone. The electrical resistance of vaginal mucus was analyzed in 20 postpartum Holstein Friesian cows by the use of a probe inserted into the anterior vagina every other day for 30 days. The correlation between milk progesterone and mucus resistance was 0.22. Mean resistance of vaginal mucus was 44 ohms for both cycling and cystic cows.

Leidl and Stolla (1976) studied a series of investigations on electronic resistance measurements of the vaginal and cervical mucus during the estrus cycle. They observed a distinct relationship between the resistance measurements and the stage of estrus cycle in cows. The lowest values were recorded during estrus and pathological condition of genital organs, such as follicular cysts and endometritis resulted in values corresponding to those observed during estrus.

Bohme and Buchholz (1977) reported that the electrical resistance in synchronized heifers and young cows was low on the day of estrus followed by increase in the other phases of estrus cycle.

Bobrike *et al*., (1978) recorded the electrical resistance of cervico-vaginal mucus of cows and heifers to be 309 and 700 ohms during estrus and diestrus, respectively.

Foote *et al*., (1979) monitored electrical resistance of the dorsal and ventral areas of the anterior vagina separately during estrus cycle. They reported that electrical resistance was lowest on day of insemination when mean values for neutral, ventral and dorsal readings were 31.500.60, 40.60 0.70 and 42.30 1.00 ohms, respectively. They further emphasized that the electronic probe measurements were as effective as the visual observations of estrus in identifying cows suitable for insemination.

Heckman *et al*., (1979) measured change in electrical resistance in the anterior vagina associated with increased cervical mucus secretion at estrus in three groups of cycling cows. In Group I (n-29) the dorsal vaginal electrical readings were higher than the ventral readings (77% vs. 59%). In Group II (n-24) and Group III (n-62) the mean electrical resistance on day of estrus was 31.00 1.7 and 41.00 1.3 ohms, respectively.

Carter and Dufty (1980) studied the vaginal impedance with respect to the placement of probe, time of insertion and stage of estrus cycle. Vaginal impedance declined by approximately 30% during proestrus, estrus and metestrus when compaired to diestrus At dorsal, ventral and lateral vaginal fornix the readings were 73.1±7.4, 73.0 ±12.8 and 48.2 ±3.8 ohms, respectively.

Rao and Rao (1982) measured electrical resistance of vaginal mucus at the time of insemination and correlated it with pregnancy rate in buffaloes. Measurements of resistance of vaginal mucus were taken in 584 buffaloes presented for insemination. They observed correlation between low resistance and higher pregnancy rate i.e. 66.60% and 60.00%, when insemination was done at vaginal electrical resistance of 20-25 and 26-30 ohms, respectively.

Gupta and Mishra (1982) examined the electrical resistance during estrus cycle in seven crossbred cows. The observations were taken from one estrus to another. They observed that electrical resistances during estrus cycle vary considerably and was lowest at the time of estrus and averaged 29.7 ohms. The electrical resistance thereafter continued rising and the mean ranged between 38.5 to 50.6 ohms during day 4 to day 16 of estrus cycle. Thereafter the mean electrical resistance exhibited a decline with the approach of next estrus.

Aboul-Ela *et al.*, (1983) studied association between changes in intravaginal electrical resistance and in-vitro measurement of vaginal mucus resistivity of cattle. Both criteria fluctuated during estrus cycle and fell to a minimum at estrus. The fall in resistivity was greater than that of vaginal resistance.

Singh and Kharche (1984) conducted a study on 56 normally cycling Holstein Friesian and Tharparker crossbred cows. They observed that in intense, intermediate and weak estrus groups the average electrical conductivity was 2.33± 0.21, 3.05 ± 0.39 and 3.08±0.33 Ohms/cm respectively and the ratio of estrogen and progesterone affected the degree of dissociation of molecules and the interaction of ions thereby altering the electrical conductivity.

Abdel Rahim and Nazier (1987) conducted an experiment in cows for detection of estrus by measuring electrical resistance of vaginal mucus membrane. They reported that the mean electrical resistance was 21±1.2 ohms in estrus cows and 31 ±4.8 ohms in cows not in estrus. Insemination on basis of such electrical resistance resulted in conception rate of 94%.

Smith *et al*., (1989) measured electrical conductivity obtained from electrodes that had been surgically implanted in mucosa of the vagina or in the submucosa of vulva in dairy cows, to evaluate the changes associated with the occurrence of estrus They observed that the conductivity increased significantly from estrus to non-estrus period in both vaginal and vulvar tissue.

Narasimha Rao (1991) reported that the vaginal electrical values in pregnant animals were 88-108 ohms with an average of 78 ± 3.6 ohms. The values were significantly lower for proestrus and estrus period. Measurement of vaginal electrical resistance at 20 cm depth was more reliable then at 25cm depth.

Kitwood *et al*., (1993) described a method to detect estrus by using vaginal mucus impedance. They confirmed that the positioning of vaginal probe impedance measurement is critical and it is necessary to find the lowest value of impedance in vaginal tract. The lowest recordings of the fixed point measurement occurred at 15 and 20 cm, which were significantly lower than the other fixed point measurements.

Hulsure and Pargaonkar (1995) reported that the mean Estrone.(electronic probe) readings of cows and heifers taken during different stages of estrus cycle were 127.80 ±6.10, 88. 33 ±0.70, 134.91 ±3.88 and 145.50 ±2.56 ohms during proestrus, estrus, metestrus and diestrus, respectively. The mean Estrone reading at the time of insemination was 86.50 ±0.76 ohms. A positive correlation between low Estrone readings and cow/heifer exhibiting heat was observed

Gupta and Purohit (2001) studied the changes in vaginal electrical resistance (VER) during different stages of estrus cycle and reported that the mean VER during estrus, diestrus, proestrus and anestrus was 32.68 ± 0.46, 41.26 ±1.17, 50.23 ±0.55, 43.20 ±0.64 and 55.86 ± 0.57 ohms, respectively. They also reported that insemination at lower VER distinctly improves conception rate in buffaloes (81.40% vs 16.66%) with 26 and 40 ohms, respectively.

Meena *et al*., (2003) conducted an experiment to detect efficiency of vaginal electrical resistance measurement for estrus detection in cows. They observed mean vaginal electrical resistance during estrus, metestrus, diestrus, proestrus and anestrus was 32.68±0.46, 41.26±1.17, 50.23±0.55, 43.52±0.54 and 55.86±0.57 ohms, respectively.

Sharma *et al*., (2004) conducted an experiment to observe the pattern of vaginal mucus impedance (VMI) during estrus. They reported that VMI at 0, 6, 12, 18 and 24 h after onset of estrus were 41.2 ±0.76, 38.5 ±1.38, 33.7 ±0.83, 42.25 ± 0.76 and 50.3 ± 0.58 ohms, respectively. The lowest VMI was recorded 12 h after onset of estrus. They further reported a significant association between VMI and pregnancy rates. The pregnancy rates at day 60 were 63.15 and 33.33% in cows with VMI of 25-34 (low) and 35 and above ohms (high), respectively.

Zuluaga *et al.,* (2008) conducted an experiment to study whether vaginal electrical resistance (VER) could be used as an indicator of follicular maturity and suitability for timed artificial insemination in beef cows subjected to a synchronization of ovulation protocol (Co-Synch plus CIDR) and reported that mean VER (ohms) was greatest (101.4 ±0.8) on day 0 and declined to 95.2 ±0.8 and 82 ± 0.8, respectively on day 7 and day 10.

2.5 Duration of estrus

Villa-Godoyet *et al.,* (1990) determined effects of energy balance and body condition on estrous behavior and estrous cycles in Holstein heifers. Before the experiment heifers were fed so body condition remained moderate or they became fat. During the 2 x 2 factorial experiment, moderate and fat heifers were in positive or negative energy balance. Heifers were fed individually twice daily, and energy balance was calculated daily. Heifers were observed for 30 min every 3 b, and all standing and mounting events were recorded for three consecutive estrous cycles. During the experimental phase, EB and BC, as main effects, did not affect duration of estrous cycles (22.4 ± .7 d), duration of diestrus (15.3 ± 1.1 d.), or intervals from onset of estrus to onset of diestrus (5.2 ± .5 d in).

Stevenson *et al*., (1995) studied the effectiveness of two estrus-detection methods (visual observation and radiotelemetric, pressure-sensitive, rump-mounted devices) was compared in peripubertal, crossbred yearling beef heifers. Heifers (n = 50) were fitted with a pressure-sensitive device affixed to their rumps to which a battery operated radio transmitter was connected. Number of standing events during estrus, determined by the radiotelemetric device, averaged 50.1 ± 6.4 per heifer, with the duration of estrus ranging from 2.6 to 26.2 h (average = 14 ± .8 h). Visual observation failed to detect 11 of 41 heifers (37%) that were detected by the radiotelemetric device. Heifers with fewer standing events (19.3 vs 60.5; P < .001) and estrus of shorter duration (8.4 vs 15.6 h; $P < .001$) were those not identified by visual observation.

Richardson *et al*., (2002b) determined fertility of heifers after synchronization of estrus using PGF2α, preceded by progesterone (P4), GnRH, or both, and to examine the variability of estrual characteristics in heifers before first and second AI. Duration of estrus was recorded 12 ± 0.6 and 11 ± 0.6 for 1st and 2nd AI respectively.

Sathiamoorthy and Subramanian, (2003) observed that the duration of estrus was found to be 20.5±2.5hours in 60 days postpartum crossbred cows treated with Ovsynch protocol and the induced estrus was recorded as 90per cent.

Ghuman *et al*., (2011) reported the average duration of estrus exhibited by cattle as 59.33±2.99h and no abnormality in estrual cervico vaginal mucus was recorded.

Velladurai *et al.*, (2014) observed the pattern of induced estrus and conception rate following Ovsynch programme in postpartum dairy cows and reported the duration of estrus in Ovsynch treated group was recorded as 29.84±0.67h and in control group as 28.78±0.67h.

Silper *et al.*, (2015) observed two activity monitoring systems—Heatime (SCR Engineers Ltd., Netanya, Israel) and IceTag (IceRobotics Ltd., Edinburgh, UK)—were compared on their ability to detect and quantify estrus expression. Holstein heifers (n = 57) were fitted with Heatime (HT) and IceTag (IT) sensors from 12 mo of age until confirmation of pregnancy. Estrus duration was recorded by HT as 14.3 ± 4.1 h [mean ± standard deviation (SD)] and by IT as 15.0 ± 4.0 h;

LingaSwamy, (2017) reported 90.00 and 100.00% in crossbred cows estrus response, mean onset of estrus was in 51.80±1.07 and 50.90±1.20 hrs mean duration of estrus in crossbred cows was 21.90±0.69 and 22.30±0.79hrs in Ovsynch and Ovsynch+GnRH groups, respectively

2.6 Estrus Synchronization response

Tegegne *et al*., (1989) synchronized heifers with PGF2α, PRID and SMB and recorded response 85.7, 68.0 and 81.5 per cent, respectively. The author mentioned overall mean response as 81.8 per cent.

Mawhinney *et al*., (1999) observed estrus response 70% of cows synchronized for estrus with Ovsynch protocol.

Lamb *et al*., (2004) observed GnRH+PGF2α protocol as effective for synchronizing estrus in replacement beef heifers. Injection of 100μg of GnRH (day 7) followed by 25mg of PGF2α (day 0) (n=160). The induction response was observed in 73% of heifers during the target breeding week.

Kasthuri, (2006) noted exhibited intense estrus as 50% and intermediate as 50% and 0% weak estrus was noticed in postpartum anestrus crossbred cows treated with Ovsynch protocol.

Ansari, (2007) recorded 80% estrus response in GnRH–PGF2α–GnRH treated postpartum anestrus crossbred cows and mean interval between treatment and onset of induced estrus found as 7.00±1.46 days and cows exhibited intense, normal and weak estrus was found as 0, 60, and 40%, respectively.

Cevik *et al*., (2010) reported 46.15% estrus response rate in Ovsynch protocol in postpartum dairy cows

Virmani *et al*., (2013) studied Ovsynch plus (PMSG-3d) protocol on 6 Sahiwal cows with the history of postpartum anestrus. The treatment was found to be 100% effective in induction of estrus.

Patel *et al*., (2013) studied the 30 postpartum crossbred cows, comprising 20 true anestrus and 10 normal cyclic cows to evaluate clinical response to estrus induction protocols and plasma progesterone and biochemical profile at different time intervals post treatment/pot-AI. The estrus induction response obtained with Ovsynch protocol in anestrus 10 cows was 90% with estrus induction intervals of 86.67±3.33hrs from PG injection, respectively.

Caraba and Velicevici, (2013) synchronized by Ovsynch protocol on 15 dairy cows. The estrus response for cows in Ovsynch protocol as 63%.

Buhecha *et al.*, (2015) observed the 12 true anestrus crossbred cows each subjected to Ovsynch protocol, 100% cows (n=12) in group exhibited behavioural estrus with prominent to moderate signs. The mean estrus induction intervals from PGF2α injection were 69.83±0.87h in protocol.

Dhami *et al.*, (2015) studied all the cows (100%) under Ovsynch protocols exhibited induced estrus with varying intensity similar to normal cyclic control group within 42-72h from the time of PGF2α injection. The mean estrus induction interval was recorded as 64.13±1.33h observed in cows under Ovsynch protocols.

Rana *et al.*, (2018) used Ovsynch with TRIU-B protocol on postpartum anestrus cows. The estrus expression rate within one week was found to be significantly higher in Ovsynch-TRIU-B treated cows 80% (P=0.003). Resumption of cyclicity or ovulation rate within two week and estrous expression rate within one month was found to be very similar between cows treated with ovsynch with TRIU-B i.e. 100%.

2.7 Intensity of estrus

Kasthuri, (2006) noted exhibited intense estrus as 50% and intermediate as 50% and 0% weak estrus was noticed in postpartum anestrus crossbred cows treated with Ovsynch protocol.

Rajeswari, (2008) observed different estrus and ovulation synchronization protocol in postpartum crossbred dairy cows and recorded the time taken for induction of estrus in Ovsynch group as 52.50 ± 0.99h from PGF2α injection and

16.67% intense, 33.33% medium and 50% low intensity of estrus in crossbred cows treated with Ovsynch protocols, with a mean duration of 37.33±0.71h.

Senthilkumar and Chandrahasan, (2015) observed synchronized cows exhibited weak, normal and intense estrus as 8.33, 58.33 and 33.33%, respectively

Ahmed *et al*., (2016) treated cows with Ovsynch protocol and reported the onset of estrus and duration of estrus was recorded as 48.750±0.713 and 21.83±0.787h and the intensity of estrus was intense, intermediate and weak as 16.67, 66.67 and 16.67% respectively.

Shahid *et al*., (2019) undertaken study to describe the duration and intensity of natural and induced estrus in indigenous heifers and cows. A total of 12 heifers and 18 cows were synchronized using the Ovsynch protocol (day 0 GnRH-1, day 7 PG, day-9 GnRH-2). They were observed for total duration of estrus and standing estrus. The primary and secondary estrus signs were observed. The total duration of heat in heifers was 18.63±1.03 and 18.00±1.58 h in induced and natural group respectively. The duration of standing estrus in induced and natural groups was 10.63±0.60 and 8.25±0.73 h, respectively. Numerically the induced group had lower total estrus intensity score (48.83±5.06) compared to natural group (59.67±5.87).

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

The present research work entitled "Fertility response of the sex sorted semen in crossbred cattle'' was carried out on 40 crossbred anoestrus heifers and cows. The research work was conducted on pubertal heifers and pluriparous crossbred cows presented at Teaching Veterinary Clinical Complex, Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Udgir, Dist Latur. Animals for experiments were also selected from private Dairy Farms in Latur and Osmanabad districts which area is under college jurisdiction and the study was completed during the period July to September 2021.

3.1 Location and Climatic Condition of the region

The present research work was conducted in two different districts of Maharashtra state. In Osmanabad district Tuljapur and Kalamb taluka is adjacent area to Western Maharashtra and Udgir in Latur district. The area is located at 643 meters above mean sea level, between 77º 07' 12.00" East longitude and 18º22'48.00" North latitude. It is located at the border of Maharashtra, Karnataka and Andhra Pradesh. The maximum temperature noted in summer season is 45.6^{0}C and minimum 10.2^{0}C in winter season. The average rainfall is 781.86 mm.

3.2 Selection of Experimental animals

Forty anoestrus crossbred cattle were selected. Animals were divided into four groups, each group carry ten animals. Group I carried ten pubertal crossbred heifers, sex sorted semen was used for insemination. Group II included ten pubertal crossbred heifers, conventional semen was used for artificial insemination. Group III was included ten crossbred cow, sex sorted semen was used for insemination. Group IV included ten crossbred cow, conventional semen was used for artificial insemination. The animals were selected on the basis of reproductive history and gynaeco-clinical examinations. Heifers and cows with satisfactory body condition score, absence of estrus and absence of any abnormal vaginal discharge were selected. All the experimental animals were screened gynaeco-clinically by performing rectal examination on the day of treatment/selection of animals and at the time of insemination. The gynaeco-clinical examination data of each case were recorded before start of research work. Selected animals were dewormed with routine drug followed by mineral supplementations @ 50 gm daily per head, inj. Urimin 10 ml and inj. Intavita-H @ 5ml intramuscularly for 3 alternate days.

Table 3.1 Research Design

Sn.	Group	Type of Animal	No. of Animals	Treatment protocol	Drug schedule	Type of semen used
1	I	Pubertal heifer	10	GnRH+PGF2α+GnRH+GnRH (GPG+G)	0 Day-GnRH @ 5ml (4mcg/ml), 7th day-PGF2α @2ml(250mcg/ml) 9th day-Inj.GnRH @2.5ml(4mcg/ml) 10th Day Inj.GnRH@2.5ml(4mcg/ml) followed by Artificial Insemination	Sex sorted semen
2	II	Pubertal heifer	10		0 Day-GnRH @ 5ml (4mcg/ml), 7th day-PGF2α @2ml(250mcg/ml) 9th day-Inj.GnRH @2.5ml(4mcg/ml) 10th Day Inj.GnRH@2.5ml(4mcg/ml) followed by Artificial Insemination	Conventional semen
3	III	Pleuriparous Cow	10		0 Day-GnRH @ 5ml (4mcg/ml) 7th day-PGF2α @2ml(250mcg/ml) 9th day-Inj.GnRH @2.5ml(4mcg/ml) 10th Day Inj.GnRH@2.5ml(4mcg/ml) followed by Artificial Insemination	Sex sorted semen
4	IV	Pleuriparous Cow	10		0 Day-GnRH @ 5ml (4mcg/ml) 7th day-PGF2α @2ml(250mcg/ml) 9th day-GnRH@2.5ml(4mcg/ml) 10th Day-GnRH@2.5ml(4mcg/ml) followed by AI	Conventional semen

Plate 3.1 Device for Vaginal Electrical Resistance

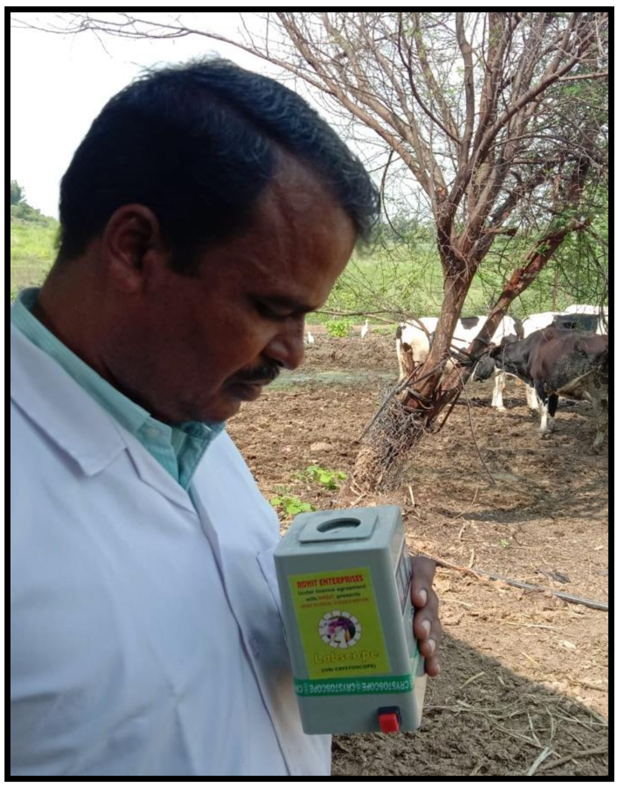

Plate 3.2 Use of Labscope on field for examination of cervical discharge

Plate 3.3 Rectal examination of crossbred pubertal heifers

Plate 3.4 Rectal examination of crossbred pluriparous cow

3.3 Details of treatment protocol used in experimental animals

All experimental animals were randomly allocated in 4 groups. Two groups of pubertal anoestrus heifers and two groups of pluriparous anoestrus cows, each group carries ten animals. The cows in treatment groups were synchronized with GPG+G protocol.

The GPG+G protocol is followed as Inj. GnRH @ 20 mcg on the day of start of synchronization program followed by inj. PGF2α @ 2 ml on day 7 and second dose of GnRH @ 10 mcg administered 48 h after PGF2α injection (day 9) and one additional dose of inj. GnRH @ 10 mcg i.m. at the time of A.I. on day 10^{th}, fixed time insemination performed 24hrs after the second GnRH injection. The same protocol was used in all groups for estrus synchronization.

3.3.1 Group I (n=10): Pubertal heifers synchronized using GPG+G and fixed time artificial insemination with sex sorted semen

All the heifers were examined per rectally to determine the functional structure present on both the ovaries CL or GF if any. Also used Draminski's electronic heat detector to know vaginal electric resistance (VER). Heifers were administered 5 ml (20 mcg) injection of Buserelin acetate **(**GnRH) by intra muscular route on day 0. On day 7, heifers were administered with 2 ml (250 mcg) injection of Cloprostenol (PGF2α) by intramuscular route. Second dose of Buserelin acetate **(**GnRH) @ 10 mcg was given on day 9, 48 h after PGF2α injection. On day 10^{th}, animal was again given one additional dose of inj. GnRH 10 mcg intra muscularly at the time of A.I. Heifers were inseminated with sex sorted frozen semen 24 h after second injection of Buserelin acetate **(**GnRH) analogue.

3.3.2 Group II (n=10): Pubertal heifers synchronized using GPG + G protocol and fixed time artificial insemination with conventional semen

All the heifers were administered with 5 ml (20 mcg) injection of Buserelin acetate **(**GnRH) by intra muscular route on day 0, after using Draminski's electronic heat detector to know vaginal electric resistance (VER). On day 7 heifers were administered with 2 ml (250 mcg) injection of Cloprostenol (PGF2α) by intramuscular route. Second dose of Buserelin acetate (GnRH) with 10 mcg dose rate was given on day 9, 48 h after PGF2α injection. One additional dose of 10 mcg GnRH was given on day 10th at the time of A.I. Heifers were inseminated with conventional semen after 24 h of second dose of Buserelin acetate (GnRH) analogue.

3.3.3 Group III (n=10): Pluriparous cows synchronized using GPG+G protocol and fixed time insemination with sex sorted semen

All crossbred cows were examined per-rectally to determine the functional structure present in the both ovaries if any. Also used Draminski's electronic heat detector to know vaginal electric resistance (VER). Cows were administered with 5 ml (20 mcg) injection of Buserelin acetate (GnRH) by intra muscular route on day 0. On day 7 cows were administered with 2 ml (250 mcg) injection of Cloprostenol (PGF2α) by intramuscular route. Second dose of Buserelin acetate (GnRH) with dose rate 10 mcg was given on day 9, 48 h after PGF2α injection. On day 10th animal was again given one additional dose of GnRH 10mcg intramuscularly at the time of A.I. Cows were inseminated with sex sorted semen 24 h after second injection of Buserelin acetate (GnRH) analogue.

3.3.4 Group IV (n=10): Pluriparous cows synchronized using GPG+G protocol and fixed time insemination with conventional semen

All crossbred cows were examined per-rectally to determine the functional structure present in the both ovaries if any. Also used Draminski's electronic heat detector to know vaginal electric resistance (VER). Cows were administered with 5 ml (20 mcg) injection of Buserelin acetate (GnRH) by intra muscular route on day 0. On day 7 cows were administered with 2 ml (250mcg) injection of Cloprostenol (PGF2α) by intramuscular route. Second dose of Buserelin acetate (GnRH) with dose rate 10 mcg was given on day 9, 48 h after PGF2α injection. On day 10th animal was again given one additional dose of GnRH 10mcg intra muscularly at the time of A.I. Cows were inseminated with sex sorted semen 24 h after second injection of Buserelin acetate (GnRH) analogue.

Plate 3.5 Use of electronic estrus detector in crossbred heifer

Plate 3.6 Use of electronic estrus detector in crossbred cattle

Plate 3.7 Estrual Cervical Discharge

Plate 3.8 Typical Fern pattern of Estrous Discharge

3.4 Managemental practices

The selected animals were kept under clean hygienic conditions at different farms viz. Private Dairy Unit, Bortala Tanda, Udgir (Dist. Latur), Borgaon and Tuljapur (Dist. Osmanabad) maintained in stall fed management as well as loose housing system. Animals were provided all essential nutrients by means of using silage @ 3 to 5 kg/day, dry jowar fodder @8 to 10 kg/day, green grasses like super Napier, maize, Lucerne etc. and mineral mixture. Experimental animals were fed a ration consisting of concentrates, roughages, mineral mixture and salt. Fresh tap water was available ad-libitum. Physical characteristics of cervical mucus (consistency, fern pattern and pH) collected during spontaneous and induced estrus were studied in animals when presented for AI.

3.5 Details of Hormones used

a) Buserelin acetate (GnRH)

Buserelin acetate is a peptide hormone and a synthetic hypothalamic releasing hormone analogue for the gonadotropins, namely LH (luteinising hormone) and FSH (Follicle stimulating hormone). The mode of action of buserelin is identical to that of natural gonadotropin releasing hormone (GnRH). As FSH and LH play a key role in the maturation of preovulatory follicle and ovulation, buserelin has the ability to induce and synchronize ovulation and improve conception rate.

i) Composition: Each ml contains 4 mcg Buserelin acetate; equivalent to Buserelin 4 mcg.

ii) Dosage and Administration: It is effective when given at 2.5 ml (10 mcg) intramuscularly.

iii) Presentation: Buserelin is available in pack of 2.5 ml, 5 ml and 10 ml vials.

b) Cloprostenol (PGF2α)

Cloprostenol is a synthetic prostaglandin analogue, structurally related to prostaglandin **(**PGF2α**)** having a specific luteolytic action. It causes functional and morphological regression of corpus luteum (luteolysis) in cattle followed by return to estrus and normal ovulation in cases of sub-estrus or anestrus.

i) Composition: Each ml contains Cloprostenol Sodium 263 mcg IP equivalent to Cloprostenol 250 mcg.

ii) Dosage and Administration: For effective synchronization of estrus it is given at 2 ml (500 mcg) intramuscularly.

iii) Presentation: single dose vial of 2 ml (500 mcg).

3. 6 Parameters to be studied

3.6.1 Estrus detection in experimental animal during induced estrus

The heifers and cows in estrus were detected by observing behavioral signs of estrus like cow allowing sniffing and licking the external genitalia by other animals, restlessness, mounting, discharge of cervical mucus, etc. in synchronized cows. Also the estrus in cows was confirmed by per-rectal examination of genitalia. The changes like relaxation of cervix, tonicity of uterus and functional structures existing in the ovaries were assessed through rectal palpation of genital organs to confirm the estrus in animals.

3.6.2 Time required for onset of estrus

Time required for onset of estrus was calculated in hours from time of $PGF2\alpha$ administration to the time of appearance of first estrus signs.

3.6.3 Intensity of estrus.

Intensity of estrus was studied on the basis of behavioral changes and gynaeco clinical observations and it was evaluated in terms of percentage as intense, intermediate and weak. Estrual symptoms were tummification of vulva, swollen and edematous vulva, cervico-vaginal mucus discharge, vulvar mucus membrane moist and congested, uterine tone and cervix opened.

3.6.4 Duration of estrus

Duration of estrus was calculated as an interval from the appearance of first estrus signs till the behavioral symptoms cease to be expressed by the animals.

Plate 3.9 AI in Crossbred Heifer

Plate 3.10 AI in crossbred cow

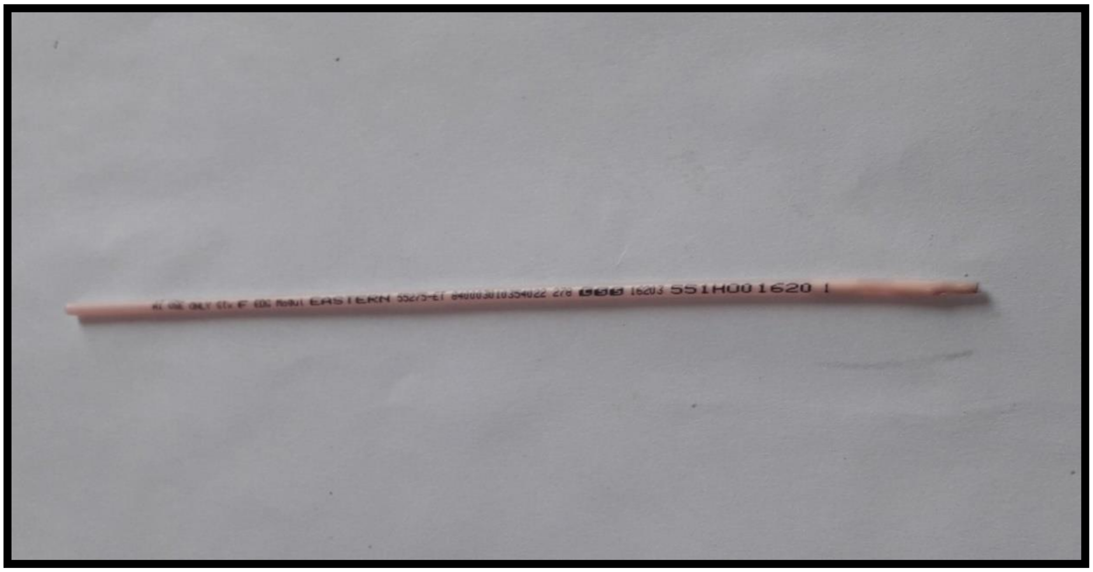

Plate 3.11 Sex sorted semen straw

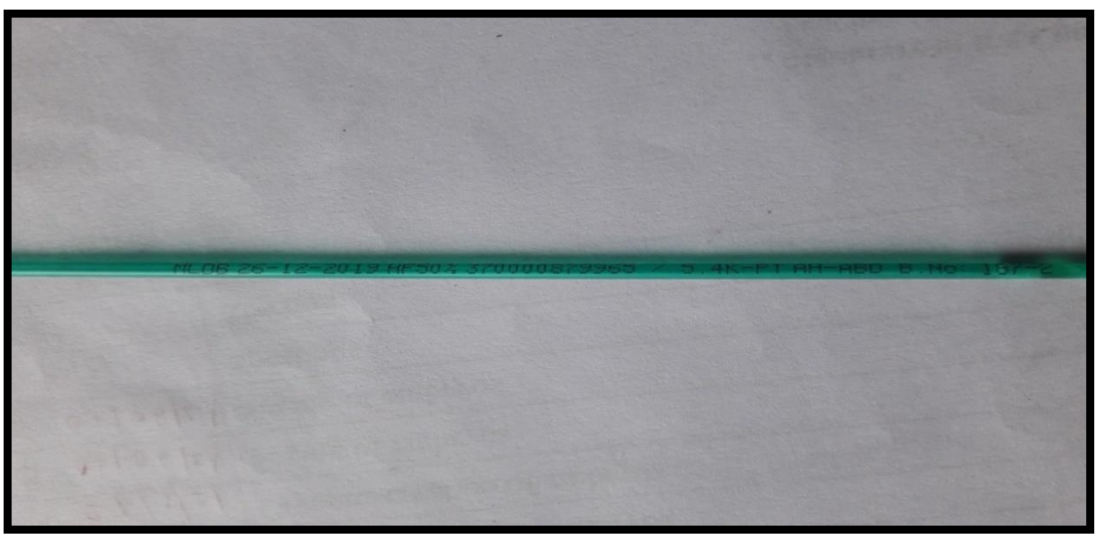

Plate 3.12 Conventional semen straw

Plate 3.13 Tummification of vulva in crossbred cow

Plate 3.14 Injecting Hormones to crossbred cow

3.7 Measurement of Vaginal Electrical Resistance

Vaginal electrical resistance was measured with the help of an electronic probe i.e. “DRAMNISKI” estrus detector (DRAMINSKI Electronics in Agriculture, Poland), DRAMNISKI readings were taken on day 0, 7 and 10 of experiment. DRAMNISKI readings were converted to Ohms (1 ohm-10 Units of DRAMNISKI reading). Procedure for measurement of electrical resistance

3.7.1 Procedure for measurement of electrical resistance

Before beginning to obtain the measurements following things should be kept ready:

1. Approximately one gallon of water in a clean bucket.

2 Tissue paper.

3. Probe disinfectant (Savlon)

4. Paper sheets for taking readings.

3.7.2 Cleaning of probe

The probe was disinfected by applying generous amount of disinfectant to a tissue paper and scrubbed vigorously over the full length of probe for about 10 seconds The disinfectant was wiped out from the probe by applying clean tissue paper by wetting in bucket of warm water thoroughly.

3.7.3 Cleaning of vulvar area

The vulvar area was scrubbed thoroughly by applying few drops of disinfectant and rinsed with clean tissue paper.

3.7.4 Insertion of probe in to vagina

Holding the probe in one hand, the vulvar lips of the animal were separated with the other hand's thumb and forefinger and probe was inserted. Insertion was continued till the pressure of cervix was felt. The probe was rotated from left to right and in and out to obtain good coating of mucus on electrodes.

3.7.5 Recording of VER readings

The probe was held stationary after insertion for 5-10 seconds and then button was switched on. The VER readings displayed on screen were recorded.

3.8 Fern Pattern

Small drop of estrual discharge was placed on grease free glass slide and thin smear was made. The smear was dried in air and examined under low power (10 X) and high power (40 X) of microscope for fern pattern. Cervical mucus with primary, secondary and tertiary branches of fern pattern were classified as typical; fern pattern with primary and secondary branches was classified as atypical; whereas nil fern pattern was noted when no primary, secondary and tertiary branches were noticed.

3.9 Details of semen straws used for artificial insemination

3.9.1 Sex sorted semen straws

The sex sorted semen used for artificial insemination in the present study was produced by BAIF Urulikanchan, Pune with 90 % of X gene sperms and 10 % of Y gene sperms. The semen was obtained from HF 100 % bull frozen in 0.25 ml French mini semen straw containing $2x10^6$ sexed spermatozoa. This technology is based on unequal DNA amounts in the X-chromosome bearing sperm versus Y-chromosome bearing sperm. The fluorescence intensity of DNA binding dye (Hoechst 33342) remains higher in X sperm than Y sperm which can be sorted using a flow cytometry. The claimed pregnancy rate of sexed semen straw is 52 % whereas conventional semen has 60 % pregnancy rate (Rai, 2018).

3.9.2 Conventional Semen Straws

Conventional frozen semen was used produced by Frozen Semen Lab., Animal Husbandry, Government of Maharashtra; Harsul, Aurangabad obtained from HF 75 % bull frozen in 0.25 ml French mini semen straw containing 20×10^6 unsexed spermatozoa.

3.10 Statistical Analysis

All reproductive parameters were recorded in the form of tables. The data was statistically analyzed by use of technique described by Snecdor and Cochran (1994).

RESULTS AND DISCUSSION

CHAPTER 4

RESULTS AND DISCUSSION

The animal husbandry sector now has been become a commercial venture in many developing and developed countries. It plays a very crucial role in national economic growth as well as livelihood generation of rural population. The commercial sexed semen technique is now considered as most advanced technique for milk and animal by-products production. By keeping in mind the importance of sexed semen technology and its role in revolutionary changes in dairy industry, present study entitled as "Fertility response of the sex sorted semen in crossbred cattle'' was designed to observe effect of estrus induction protocol in pubertal heifers and pluriparous cows by using sex sorted as well as conventional semen. Investigation of conception rate was one of the most important objectives in pubertal heifers as well as in pluriparous crossbred cows.

The present research work was under taken on twenty each crossbred pubertal heifers and pluriparous cows. The research work was carried on animals presented to Teaching Veterinary Clinical Complex, Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Udgir and Private Dairy Farms around Udgir Tahsil Dist Latur. Experimental animals were also selected from Dairy Farms in Tuljapur and Kalamb Tahsil Dist. Osmanabad. The study was carried out during the period from July 2021 to October 2021.

Selected animals were divided in four equal groups assigned to be inseminated with sex sorted semen (2×10^6 cells per inseminating dose) and to receive conventional semen (20×10^6 cells per inseminating dose) during induced estrus with GPG+G. All the experimental animals were allotted in groups as follows:

Group I (n=10): Pubertal heifers induced estrus using GPG+G protocol and fixed time insemination with frozen sex sorted semen.

Group II (n=10): Pubertal heifers induced estrus using GPG+G protocol and fixed time insemination with conventional frozen semen.

Group III (n=10): Pluriparous cows induced estrus using GPG+G protocol and fixed time insemination with frozen sex sorted semen.

Group IV (n=10): Pluriparous cows induced estrus using GPG+G protocol and fixed time insemination with conventional frozen semen.

A detailed study was carried out and conception rates in pubertal heifers and pluriparous cows were observed when subjected to sex sorted semen and conventional semen in induced estrus groups, and subsequently discussed in detail under following subheads.

4.1 Effect of estrus induction protocol in pubertal heifers with sex sorted semen and conventional semen

The objective of the present study was to evaluate the efficacy of sex sorted semen and conventional semen in crossbred pubertal heifers with the treatment of estrus induction protocol i.e. GPG+G by measuring conception rates after FTAI. During the experiment, the pubertal heifers randomly allocated in Group I and II were induced estrus using GPG+G protocol.

The Ovsynch (GPG) protocol is an effective breeding programme used in lactating dairy cows and heifers worldwide but in present study, GPG + G protocol was used to induce the animals. An additional dose of GnRH at the time of A.I. was used for prompt ovulation. The protocol involves the following three hormone administrations: a GnRH administration (GnRH-1) on day 0, followed by PGF2α administration on day 7, and another administration of GnRH (GnRH-2) 48 h later (Pursley *et al.*, 1995). The administration of these hormones synchronizes the initiation of a follicular wave by ovulation of an ovulatory follicle, the regression of the corpus luteum and eventually, the ovulation of the dominant follicle of the new follicular wave. This precise synchronization of ovulation within an 8 h period, 24–32 h after the administration of GnRH-2, allows for successful timed artificial insemination (TAI) without any estrus detection. Therefore, the success of the Ovsynch protocol dramatically depends on the responses to these hormones. With respect to the response to GnRH administrations, fertility is better in cows that respond to the GnRH-1 in the Ovsynch protocol compared to those cows that do not respond.

Estrus synchronization offers producer's opportunity to achieve acceptable AI conception rates to conventional as well as sex sorted semen without the need to visually detect estrus and adopting fixed time insemination program (FTAI). The estrus induction program in experimental cows in Group I and II consisted of administration of 5 ml (20mcg) injection of Buserelin acetate **(**GnRH) by intra muscular route on day 0. On day 7, heifers were administered with 2 ml (500 mcg) injection of Cloprostenol (PGF2α) by intramuscular route. Second dose of Buserelin acetate **(**GnRH) with dose rate of 10 mcg was given on day 9, 48 h after PGF2α injection. All responded heifers were fixed time inseminated with sex sorted frozen semen and conventional frozen semen 24 h after second injection of Buserelin acetate **(**GnRH) analogue as in subsequent groups. One more additional dose of GnRH 10 mcg was injected at the time of insemination. The pregnancy status in the animals was confirmed by per rectal examination after 60 days after insemination.

Effect of estrus induction protocol in pubertal heifers inseminated with sex sorted semen (Group I) and conventional semen (Group II) are presented in Table 4.1, 4.2, 4.3, 4.4 and fig. 4.2.

Table 4.1 Effect of estrus induction protocol in pubertal heifers

Sr. no.	Parameter		Observations	
			Group I	Group II
1	No. of animals under research		10	10
2	No. of animals responded after treatment (%)		10 (100)	8 (80)
3	Onset of oestrus after treatment (hrs)		24	24
4	Type of semen used		Sex sorted semen	Conventional semen
5	Duration of oestrus (hrs)		25.2 ± 4.54	24.60±2.27
6	Conceptions (%)	1st estrous	3/10 (30)	5/10 (50)
		2nd estrous	2/4 (50)	2/6 (33.33)
		3rd estrous	00	00
7	Overall Conception rate (%)		5 (50)	7 (70)
8	Services per conception		2.8	1.8
9	Intensity of estrus (%)			
		Intense	40	40
		Intermediate	50	50
		weak	10	10
10	Fern Pattern (%)			
		Typical	50	70
		Atypical	50	30
11	Non cyclic animals		03	02
12	No. of animals continued cyclicity		02	01

Note: All the above results calculated based on descriptive statistical analysis

Table 4.2 Overall estrual symptoms in experimental animals

Sr. no.	Estrual symptoms	Observations (%)	
		Group I	Group II
1	Tummification of vulva	90	90
2	Swollen and edematous vulva	100	80
3	Cervico-vaginal mucus discharge	100	100
4	Vulvar mucus membrane moist and congested	80	80
5	Uterine tone	100	100
6	Cervix opened	100	100

Note: All the above results calculated based on descriptive statistical analysis

Table 4.3 Duration of estrous

Parameter/ Groups	Group I (Mean ± SE)	Group II (Mean ± SE)	Group III (Mean ± SE)	Group IV (Mean ± SE)	*p value*
Estrous duration (Hrs)	25.2 ± 4.54	24.60±2.27	20.2± 1.87	30.00±2.68	0.171^{NS}

(NS = Non-significant)

Table 4.4 Duration of estrous in heifers and cows

Parameter	Heifers (Mean ± SE)	Cows (Mean ± SE)	*p value*
Estrous duration (Hrs)	24.9 ± 2.47	25.1± 1.949	0.949^{NS}

(NS = Non-significant)

In the present study, crossbred pubertal anoestrus heifers were induced estrus with GPG + G in Group I & II, 100.00 % heifers were responded within 24 hours of treatment. Duration of oestrus was noted 25.2 ± 4.54 and 24.60±2.27 hours in Group I & II, respectively. Intensity of estrous was recorded as intense, intermediate and weak as 40, 50, 10 and 40, 50, 10 per cent in Group I & II, respectively. Fern pattern were found to be typical and atypical as 50, 50 and 70, 30 per cent in Group I & II.

Pregnancy diagnosis was performed after 60 days of artificial insemination in all animals. In Group I, overall conception rate by use sex sorted semen was found in 5 (50 %) with 2.8 services per conception. In 1st estrous, 3 heifers and 2nd estrous, 2 heifers were found to be pregnant. In group II, overall conception rate by use of conventional semen were observed 7 (70 %) with 1.8 services per conception. In 1st estrous, 5 heifers and 2nd estrous, 2 heifers were found confirmed pregnant. Pubertal heifers inseminated (FTAI) with conventional semen had greater conception rate than those inseminated with sex sorted semen following induced estrus.

Statistically, it was observed that the duration of estrous across different groups was found to be non significant (p= 0.171). However, the duration of estrous in Group IV (Pluriparous cows) was longer (30.00±2.68 hrs) and shortest (20.2± 1.87 hrs) in Group III. However, duration of estrous in heifers and cows was found to be non significant (p=0.949) difference (Table 4.3 and 4.4).

A total of three heifers failed to show estrus in next subsequent estrous cycle and two heifers were not to be settled even after repeated inseminations from Group I whereas in Group II, two heifers had been shown as non cyclic and one heifer was found to be repeated tendency. The failure of conception might be cause of non infectious origin and possibly due to asynchrony of events for successful conceptions.

Estrual symptoms in Group I were recorded as tummification of vulva (90 %), swollen and edematous vulva (100%), cervico-vaginal mucus discharge (100%), vulvar mucus membrane moist and congested (80%), uterine tone (100%) and cervix opened (100%). Whereas in Group II were noted tummification of vulva (90 %), swollen and edematous vulva (80%), cervico-vaginal mucus discharge (100%), vulvar mucus membrane moist and congested (80%), uterine tone (100%) and cervix opened (100%).

After scanning of ample literature regarding the fertility parameters of heifers, very few authors have been worked on it. Lamb *et al*., (2004) observed the induction response in heifers as 73.00 per cent, which is less response than the present findings.

Tegegne *et al.*, (1989) synchronized heifers with $PGF_2\alpha$, PRID and SMB and recorded response 85.7, 68.0 and 81.5 per cent, respectively. The author mentioned overall mean response as 81.8 per cent, GPG+G found to be more successful induction protocol than other hormonal protocols in heifers as reported in the present study.

Villa-Godoy *et al.* (1990), Stevenson *et al.* (1996) and Silper *et al* (2015) who reported average duration of estrus as 22.4 ± 0.7, 14 ± 0.8 and 15.0 ± 4.0 hrs in heifers.

Richardson *et al.* (2002a) determined fertility of heifers after synchronization of estrus and reported the duration of estrus as 12 ± 0.6 and 11 ± 0.6 for 1^{st} and 2^{nd} estrus, respectively.

Shahid *et al.* (2019) synchronized heifers using Ovsynch protocol and noted the duration of heat as 18.63±1.03 hrs. Also authors were reported as numerically the induced group had lower total estrus intensity score (48.83±5.06) compared to natural group (59.67±5.87).

The present findings are close agreement with Guner *et al.* (2021) who studied comparison of pregnancy per AI of heifers inseminated with sex-sorted or conventional semen after oestrus detection or timed artificial insemination. The mean duration of oestrus was 18.6 ± 0.1 hr, Overall P/AI was 68.1% for conventional and 48.9% for sex-sorted semen regardless of insemination strategies. Thomas *et al.*, (2016) found similar reports as in the present findings for heifers inseminated with conventional semen (60%) compared to sex-sorted semen (52%).

Bodmer *et al.*, (2005) recorded 33.3% (9/27) and 59.3% (16/27) in heifers inseminated with 2x 10^6 x-bearing, frozen-thawed sperm which is less percentage than the present study. Similarly low results were noted by Healy *et al.*, (2013) as conception rates of 31.6 and 39.6 % were achieved for sexed and unsexed semen respectively.

Noonan *et al* (2016) observed in first-service conception rates were 40.3% and 56.0% for sex-sorted and conventional semen, respectively.

Colazo and Mapletoft, (2017) reported as P/AI were 69.2% (74/107) and 64.1% (75/117) for conventional and sex-selected semen (P. 0.05) which is higher than the present study.

The data indicated that artificial insemination with sex sorted semen resulted in decreased pregnancy rate in pubertal heifers than the insemination with conventional semen subjected to synchronization and FTAI program. The possible factors influencing pregnancy rates after FTAI following estrus synchronization treatment are exhibition of premature estrus, follicular growth, synchronized ovulation and also attributed to environmental conditions, may be adverse effect of sorting process by flow cytometry cause damage to DNA of spermatozoa, managemental practices followed at different places and influence of the handler examining animals. Overall conceptions were affected by various factors like insemination method, technician, climatic changes, health of heifers and many other factors.

Practical use of sexed sperm by commercial dairy herds depends on several factors, but fertility and accuracy of sex selection (i.e., purity) are probably the most important traits for evaluating the reliability of the technology. Lower pregnancy rates after insemination with sexed sperm have been reported for several species (Seidel and Garner, 2002) but data for the fertility of sexed sperm when used under field conditions are still scarce.

4.2 Effect of estrus induction protocol in pluriparopus cows with sex sorted semen and conventional semen.

All pluriparous cows were examined per-rectally to determine normal genital structure. “DRAMNISKI" electronic heat detector were also be used to know vaginal electric resistance (VER) on day 0, 7, 10, respectively. Cows were administered with 5 ml (20 mcg) injection of Buserelin acetate (GnRH) by intramuscular route on day 0. On day 7 cows were administered with 2 ml (500 mcg) injection of Cloprostenol (PGF2α) by intramuscular route. Second dose of Buserelin acetate (GnRH) with dose rate 10 mcg was given on day 9, 48 h after PGF2α injection. On day 10^{th} animal was again given one additional dose of GnRH 10 mcg intramuscularly at the time of artificial insemination. Cows were inseminated with sex sorted frozen semen or conventional frozen semen, 24 h after second injection of Buserelin acetate (GnRH) analogue.

Effect of estrus induction protocol in crossbred pluriparous cows inseminated with sex sorted semen (Group III) and conventional semen (Group IV) are presented in Table 4.3, 4.4, 4.5, 4.6 and fig. 4.2.

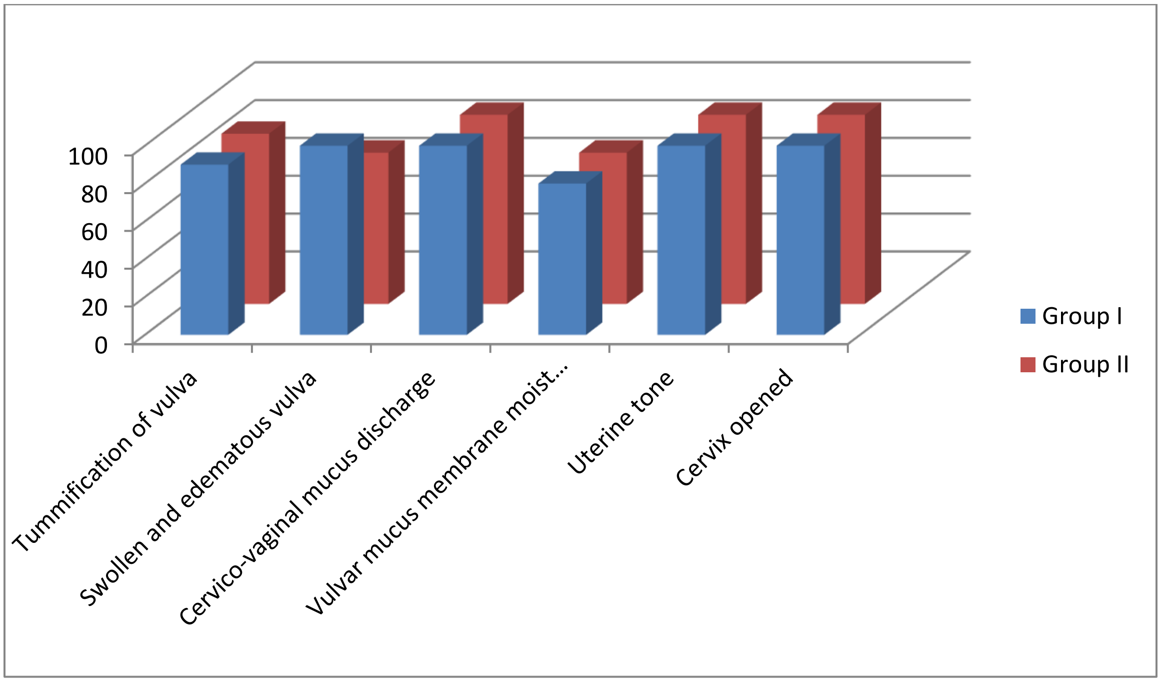

Fig 4.1 Overall estrual symptoms in experimental animals in Group I and II

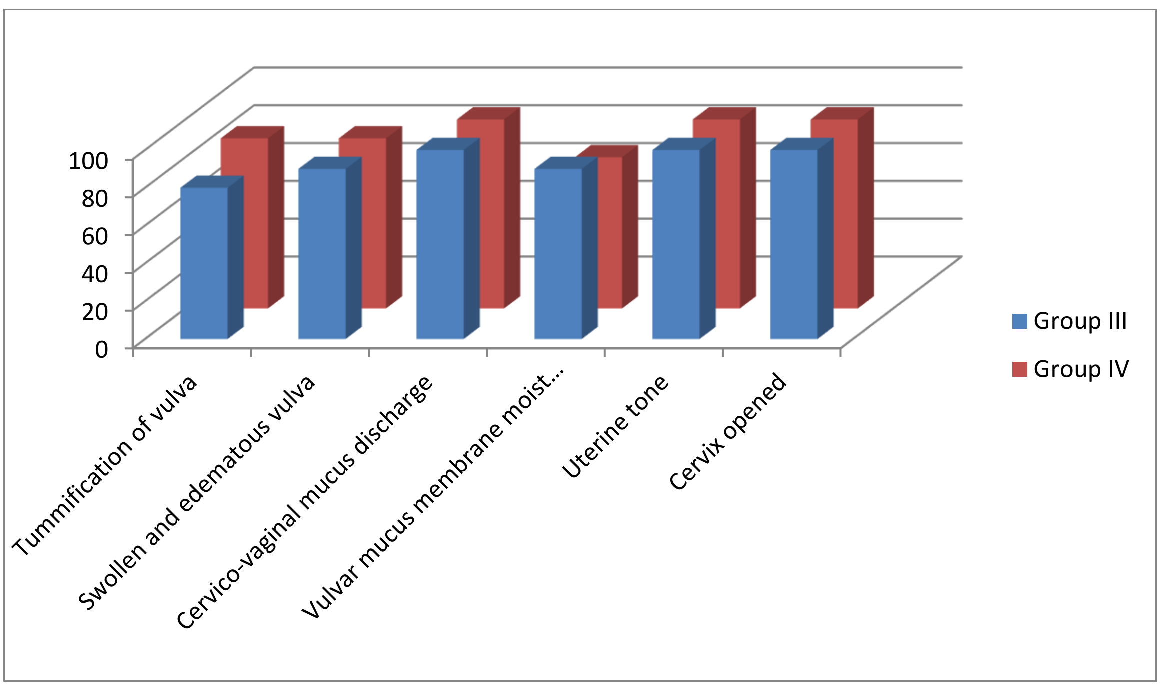

Fig 4.2 Overall estrual symptoms in experimental animals in Group III and IV

Table 4.5 Effect of estrus induction protocol in Pluriparaous cows

Sr. no.	Parameter	Observations	
		Group III	Group IV
1	No. of animals under research	10	10
2	No. of animals responded after treatment (%)	10 (100)	10 (100)
3	Onset of oestrus after treatment (hrs)	24	24
4	Type of semen used	Sex sorted semen	Conventional semen
5	Duration of oestrus (hrs)	20.2± 1.87	30.00±2.68
6	Conceptions (%) 1st estrous	4/10 (40)	2/10 (20)
	2nd estrous	00	6/7 (85.71)
	3rd estrous	3/4(75)	00
7	Overall Conception rate (%)	7 (70)	8 (80)
8	Services per conception	2.57	2.25
9	Intensity of estrus (%)		
	Intense	60	70
	Intermediate	40	30
	weak	00	00
10	Fern Pattern (%)		
	Typical	70	80
	Atypical	30	20
11	Non cyclic animals	02	01
12	No. of animals continued cyclicity	01	01

Note: All the above results calculated based on descriptive statistical analysis

Table 4.6 Overall estrual symptoms in experimental animals

Sr. no.	Estrual symptoms	Observations (%)	
		Group III	Group IV
1	Tummification of vulva	80	90
2	Swollen and edematous vulva	90	90
3	Cervico-vaginal mucus discharge	100	100
4	Vulvar mucus membrane moist and congested	90	80
5	Uterine tone	100	100
6	Cervix opened	100	100

Note: All the above results calculated based on descriptive statistical analysis

In the present study, crossbred pluriparous anoestrus cows were induced estrus with GPG + G in Group III & IV, 100.00 % cows were responded within 24 hours of treatment. Duration of oestrus was noted 20.2± 1.87 and 30.00±2.68 hours in Group III & IV, respectively. Intensity of estrous was recorded as intense, intermediate and weak as 60, 40, 00 and 70, 30, 00 per cent in Group III & IV respectively. Fern pattern were found to be typical and atypical as 70, 30 and 80, 20 per cent in Group III & IV, respectively.

Pregnancy diagnosis was performed after 60 days of artificial insemination in all animals. In Group III, overall conception rate by use sex sorted semen was found in 7 (70 %) cows with 2.57 services per conception. In 1^{st} estrous, 4 cows and 3^{rd} estrous, 3 cows were found confirmed pregnant. In group IV, overall conception rate by use of conventional semen were observed 8 (80 %) with 2.25 services per conception. In 1^{st} estrous, 2 cows and 2^{nd} estrous, 6 cows were confirmed pregnant. Pluriparous cows inseminated (FTAI) with conventional semen had greater conception rate than those inseminated with sex sorted semen following induced estrus.

Out of 10 pluriparous cows from Group III, 2 cows non cyclic and one pluriparous cows were observed cyclic repeat breeder whereas in Group IV, one each pluriparous cow was found as non cyclic and cyclic repeat breeder.

Estrual symptoms in Group III were recorded as tummification of vulva (80%), swollen and edematous vulva (90%), cervico-vaginal mucus discharge (100%), vulvar mucus membrane moist and congested (90%), uterine tone (100%), cervix opened (100%) whereas in Group IV were noted tummification of vulva (90 %), swollen and edematous vulva (90%), cervico-vaginal mucus discharge (100%), vulvar mucus membrane moist and congested (80%), uterine tone (100%) and cervix opened (100%).

The conception rate in pluriparous cows subjected to sex sorted semen (Group III; n=10) and conventional semen (Group IV; n=10) were recorded as 70 % and 80%, respectively after estrus induction with GPG+G protocol.

The cows inseminated with sex sorted semen yielded reduced pregnancy rates than with conventional non sorted semen during the experiment.

The present findings are close agreement with Dhami *et al.*, (2015) who noted 100.00 per cent response in cows after receiving Ovsynch protocol and also recorded intensity of induced estrus which is similar to normal cyclic control group within 42-72 h from the time of PGF2α injection. The author also recorded 64.13±1.33h the mean estrus induction interval with conception rates at induced estrus of 50% the overall of three cycles as 80% in cows. Difference in duration of estrus was found to be greater than the present finding; it might be due to the species specific relation, productive traits and environmental factors which alter the duration of estrous.

Similar estrus induction protocol (GPG+G) was used by LingaSwamy (2017) reported 100.00% in crossbred cows estrus response, mean onset of estrus was in 50.90±1.20 hrs mean duration of estrus in crossbred cows was 22.30±0.79 hrs. The overall conception rate was mentioned as 50.00 in Ovsynch+GnRH protocol.

Belure *et al.*, (2021) recorded the estrus induction response as 100.00 per cent. The author was also noted 25.00, 41.66 and 33.33 per cent, intense, intermediate and weak intensity of estrus, respectively. The duration of estrus was mentioned 15.32±0.13 hrs. Fern pattern was found Typical (50.00%) and atypical (50.00%) after receiving Ovsynch protocol in crossbred cows. The conception rate was found to be 50.00 per cent, these report are found very closer with the findings of the present study.

Sathiamoorthy and Subramanian, (2003) found 90.00 per cent induced estrus and the duration of estrus as 20.5±2.5 hours in postpartum crossbred cows with Ovsynch protocol.

Rana *et al.*, (2018) also tried by use of Ovsynch with TRIU-B protocol on postpartum anestrus cows and found 80 % response in treated cows. The author recorded as 100 % resumption of cyclicity or ovulation rate within two week and estrous expression rate within one month, this results are similar to the present study.

Buhecha *et al.*, (2015) observed in 12 true anoestrus crossbred cows 100.00 per cent response to Ovsynch protocol and also recorded exhibited behavioral estrus with prominent to moderate signs. The author further mentioned the mean estrus induction intervals from PGF2α injection as 69.83±0.87h in protocol, which is greater value than the present study. However, Virmani *et al.*, (2013) used Ovsynch plus (PMSG-3d) protocol in Sahiwal cows and found 100.00 % response.

Patel *et al.*, (2013) induced estrus with Ovsynch protocol in anestrus 10 cows and obtained 90.00 % response with estrus induction intervals of 86.67±3.33 hrs from PG injection, respectively.

Cevik *et al.*, (2010), Caraba and Velicevici, (2013) and Mawhinney *et al.*, (1999) synchronized cows by Ovsynch protocol and recorded estrus response as 46.15, 63.00 and 70.00 per cent, which is very lesser than the present study.

Similar type of intensity of estrus are recorded by the various authors viz. Kasthuri, (2006) found intensity response in postpartum anestrus crossbred cows treated with Ovsynch protocol as intense estrus (50.00 %), intermediate (50.00 %) and (0.00 %) weak estrus.

Ansari, (2007) recorded 80.00 % estrus response, mean interval between treatment and onset of induced estrus as 7.00±1.46 days and exhibited intense, normal and weak estrus as 0, 60, and 40%, respectively in GnRH–PGF2α–GnRH treated postpartum anestrus crossbred cows.

Velladurai *et al.*, (2014) observed 29.84±0.67 hours as duration of estrus in Ovsynch treated postpartum dairy cows. Whereas Ghuman *et al.*, (2011) reported as 59.33±2.99hours, these much differences in duration of estrus attributed to various factors but its variable reports are noted by various authors.

Ahmed *et al.*, (2016) used Ovsynch protocol in cows and reported the onset of estrus and duration of estrus as 48.750±0.713 and 21.83±0.787 hrs. Also the authors reported the intensity of estrus as intense, intermediate and weak 16.67, 66.67 and 16.67%, respectively.

Rajeswari, (2008) synchronized with Ovsynch postpartum crossbred dairy cows and recorded the time taken for induction of estrus as 52.50 ± 0.99 hrs and 16.67 % intense, 33.33 % medium and 50 % low intensity of estrus and mean duration of estrus as 37.33±0.71hrs .

Kasthuri, (2006) recorded intensity of estrus as 50.00, 50.00 and 0.00 per cent intense estrus, intermediate and weak estrus respectively in postpartum anestrus crossbred cows with Ovsynch protocol. Senthilkumar and Chandrahasan, (2015) observed synchronized cows exhibited weak, normal and intense estrus as 8.33, 58.33 and 33.33%, respectively.

Parikh *et al.*, (2018) reported 46.81 per cent estruses had arborisation pattern either typical or atypical in postpartum Gir cows. Also authors opined estrus cows with clear appearance of mucus and typical arborisation pattern had higher conception rate, similar reports were found in the present study.

Similar results are quoted by Crites *et al*., 2018 in beef cattle after fixed-time artificial insemination using either sexed ultra™ 4m sex-sorted semen or conventional semen and recorded conception rate for estrual females (62.8%). Fricke *et al*., (1998) who recorded 85% conception rate in lactating cows after receiving Ovsynch protocol. Belure *et al.* (2021) who observed the time interval between treatment and estrus as 48.28±0.72 hrs after receiving Ovsynch protocol in cows which is longer time interval as compared with the present findings.

Ansari, (2007) recorded 60% first service conception rate and overall conception rate was found to be 90% in GnRH–PGF2α–GnRH treated cows which results are closely agreed with the present study. Sharma *et al*., (2018) who observed conception rate for conventional semen as 49.32% and for sex sorted semen it was 40.00 % which is closely agreed with the present study for 1st estrus insemination in both groups.

Maicas *et al*., (2019) studied fertility of fresh and frozen sex-sorted semen in dairy cows and heifers in seasonal-calving pasture-based herds. Cows inseminated with conventional semen had greater P/AI (48.00 %) than cows inseminated with SS, irrespective of treatment.

Cevik *et al.*, (2010) reported comparison of pregnancy rates after timed artificial insemination in Ovsynch protocol in dairy cows and pregnancy rates after first AI for Ovsynch groups were observed as 76.92 %. In the case of the second AI pregnancy rates of Ovsynch group were 100.00 %,.

Bartolome *et al*., (2005) reported pregnancy rates on days 27, 45 and 90 were 25.2%, 17.5% and 13.9% for Ovsynch protocol

Keister *et al*., (1999) reported 70 % pregnancy rate in cows following Ovsynch protocol.

Roy and Twagiramungu, (1997), Geary *et al.*, (1998) and Deshmukh *et al*., (2010) quoted as 61, 54 and 50.00 per cent conception rates by use of conventional semen in cows treated with GnRH–PGF2α–GnRH protocol, which is less than the result of present study.

Karakaya-bilen *et al*. (2018) and Karakaya *et al*. (2014) used sex sorted semen (SS) containing 2.2 x 10^6 morphologically normal and 1.9 x 10^6 progressively motile sperm cells/straw and recorded conception rate as 38.1 and 31.8 per cent, resulted less results than the present findings.

Doyle *et al.* (1999) implemented synchronization program in Angus cows by using GnRH and PGF2α treatments. Cow received half of 1 x 10^6 frozen thawed sorted sperms and few cows received half of 5 x 10^5 cooled-to-18°C sorted sperm deposited into each uterine horn 6 to 26 h after observed estrus. The pregnancy rates recorded were 23 % and 25 % respectively.

Dantre and Kumar, (1999) treated with Receptal yielded 85.71% heifers expressing estrus within 23.5±1.89 days post treatment with 66.66% conception rate and conception rates of cyclic Ovsynch- treated cows (59%).

Thompson *et al*., (1999) reported in cattle synchronization with Ovsynch is associated with pregnancy rates of between 30-40% although pregnancy rates of 20 % have also been reported.

Whisnant *et al*., (1999) observed synchronization protocols and found 34.9 and 34.9%, conception and pregnancy rates respectively in dairy cattle. Conception and pregnancy rates from breeding at detected estrus after PGF2α averaged 37.7 and 19.8%, respectively.

Stevenson *et al*., (1999) undertook worked on 1,308 Holstein cows synchronized using ovsynch protocol. They found conception rate as 22.1 % in animals.

Vasconcelos *et al.,* (1999) reported that the overall synchronization rate in cows using Ovsynch protocol was 85 % and there were clear differences in response according to the day of protocol initiation. They have also recorded that 64 % of cows ovulated to the first GnRH.

Kim *et al.,* (2003) evaluated pregnancy rates in lactating Holstein cows treated with an Ovsynch protocol The Pregnancy rate after TAI following this protocol was observed as 20.6 %.

Tenhagen *et al.*, (2003) implemented synchronization protocol on 136 Holstein Frisian cows using Buserelin (GnRH analogue). The overall synchronization rate (proportion of cows with ovulation within40 h after GnRH) was 86.8 % in primiparous and 88.2 % in multiparous cows, respectively Conception rates were numerically higher in primiparous than in multiparous cows (39.7 % vs. 29.4 %, $p > 0.05$). But in our research conception rate in primiparous is lesser than pluriparous cows.

Kasthuri, (2006) recorded conception rate in GnRH–PGF2α–GnRH treated postpartum anestrus crossbred cows as 40.00 per cent in first service conception, which is also closer to our results.

Ali and Fahmy, (2007) observed the ovarian dynamics and progesterone concentrations in cyclic and non-cyclic buffalo-cows during Ovsynch program and observed lower conception rate in non-cyclic cows (37.5%) than cyclic cows (60%).

Dagli, (2009) studied 6 HF crossbred cows at average 180 days postpartum period with injection Receptal 5ml intramuscular on day 1st, injection Lutalyse 5ml intramuscular on day 7th and repeat dose of inj. Receptal on day 9th with fix timed insemination on day 11th morning. The establishment of cyclicity in 100.0% and conception rate in 33.0% cows with Ovsynch protocol.

Muneer *et al*., (2009) reported the number of services required per conception in anestrus postpartum crossbred cows treated with Ovsynch and control groups as 1.44 and 1.66 respectively.

Ravikumar *et al*., (2009) obtained ovulatory response and first service conception rates in buffaloes were 83.33% and 33.33% with Ovsynch and 100.00%

and 41.66% with Sa Filho *et al*., (2010) observed pregnancy rate of 51.4% by using progesterone based estrus synchronization protocol in Bos Indicus cows.

Caraba and Velicevici, (2013) using Ovsynch protocol versus Cosynch Protocol in Dairy Cows. They studied on 15 dairy cows which were synchronized by Ovsynch and Co synch programs. The estrus response for cows in Ovsynch protocol was of 63%. Pregnancy per insemination at 60 days was of 25%.

Barolia *et al*., (2016) studied comparative study of co synch and ov synch protocol on fertility in repeat breeder gir cow Present study was done on organized college farm. Pregnancy rate recorded for Co synch, Ovsynch and control group were 50%, 66.66%, 33.33% respectively.

Khalil and Hussein (2019) reported the conception rate by insemination at standing heat results in a similar or higher pregnancy rate compared with FTAI. In this study, a total of 8944 inseminations were included. All cows were subjected to Pre synch ovsynch protocol. Cows detected in estrus (n=7424) were artificially inseminated, whereas cows not observed estrus were submitted to FTAI. They observed conception rate was 36.64 ±1.32 using FTAI.

Higher conception rates were achieved with conventional semen than with sex sorted semen among the pluriparous cows in induced estrus. The cows inseminated with sex sorted semen are likely to be resulting in lower conception rate, since the flow cytometric cell sorting process may create potential damage to DNA of the sperm because of chemical and physical manipulations that limit sperm longevity in the female tract.

4.3 Measurement of Vaginal Electrical Resistance (VER)

Vaginal electrical resistance (VER) was measured with the help of an electronic probe i.e. DRAMNISKI" estrus detector (DRAMINSKI Electronics in Agriculture, Poland), DRAMNISKI readings were taken on day 0, 7 and 10 during research period. DRAMNISKI readings were converted to Ohms (1 ohm = 10 Units of DRAMNISKI reading). Several electronic devices have been developed to alleviate the need for visual estrus detection in cows and to provide a precise determination of the onset of estrus. Commercially available electronic technologies for estrus detection are based on changes in physical activity (pedometers), changes in electrical and chemical properties of the reproductive tract (intravaginal resistance probes), or mounting activity (mount detectors) (Rorie *et al*., 2002). The vaginal electrical resistance fluctuates with stage of the estrous cycle, is highest during the luteal phase, and declines during the follicular phase. The lowest VER values are correlated with the preovulatory LH peak (Schams *et al*, 1977, Canfield and Butler, 1989).

In present research work, it was observed different values of vaginal electric resistance (VER) at various stages of induced estrus in pubertal heifers and pluriparous cows. The values of VER were recorded as shown in table 4.7, 4.8, 4.9, 4.10 and fig 4.3 & 4.4.

Table 4.7 Average value of electronic heat detector (ohm**)**

	Group I (Mean ± SE)	Group II (Mean ± SE)	Group III (Mean ± SE)	Group IV (Mean ± SE)	*p-value*
0 day	35.10 ± 2.56	28.30±2.71	30.10±1.33	27.80±1.51	0.078^{NS}
7 day	34.10 ± 3.67^{a}	24.80 ± 1.11^{ab}	28.30 ± 1.38^{ab}	20.20 ± 1.82^{b}	0.001^{**}
10 day	29.80 ± 2.05^{a}	21.60 ± 1.10^{b}	18.90 ± 1.61^{b}	21.00 ± 0.96^{b}	0.000^{**}

(Note: **Significant at 1% level; NS = Non-significant; Means bearing different superscript different significantly from each others; SE = Standard error)

Table 4.8 Difference amongst average value of electronic heat detector in heifer and cow

Sr. No.	**Type of animal**	**Mean ± SE**	***P value***
1	Heifer	31.70 ± 1.97	0.223^{NS}
2	Cow	28.95 ± 1.01	

(NS = Non-significant)

Table 4.9 Value of electronic heat detector in pregnant and non pregnant animals (Group wise)

Days	Animals	Group I (Mean ± SE)	Group II (Mean ± SE)	Group III (Mean ± SE)	Group IV (Mean ± SE)	*P value*
0 day	Pregnant	36.60±2.40	28.00±2.77	30.00±1.63	29.13±1.56	0.066
	Non pregnant	33.60±4.76	29.00±7.51	30.33±2.85	22.50±0.50	0.606
7 day	Pregnant	35.00±6.10^{a}	25.00±1.46^{ab}	26.71±0.97^{ab}	20.75±1.96^{b}	**0.013***
	Non pregnant	33.20±4.81	24.33±1.86	32.00±3.51	18.00±6.00	0.185
10 day	Pregnant	27.00±3.13^{a}	21.14±1.39^{ab}	17.29±1.92^{b}	21.75±1.03^{ab}	**0.015***
	Non pregnant	32.60±2.29^{a}	22.67±2.03^{ab}	22.67±1.76^{ab}	18.00±1.00^{b}	**0.006****

*(Note: **Significant at 1% level; *Significant at 5% level; NS = Non-significant; SE = Standard error)*

Table 4.10 Value of electronic heat detector in pregnant and non pregnant animals (Pregnant- Non pregnant wise)

Days	Animals	Group I	Group II	Group III	Group IV
0 day	Pregnant	36.60±2.40	28.00±2.77	30.00±1.63	**29.13±1.56**
	Non pregnant	33.60±4.76	29.00±7.51	30.33±2.85	**22.50±0.50**
	P value	0.589^{NS}	0.908^{NS}	0.925^{NS}	**0.003****
7 day	Pregnant	35.00±6.10	25.00±1.46	26.71±0.97	20.75±1.96
	Non pregnant	33.20±4.81	24.33±1.86	32.00±3.51	18.00±6.00
	P value	0.822^{NS}	0.789^{NS}	0.283^{NS}	0.738^{NS}
10 day	Pregnant	27.00±3.13	21.14±1.39	17.29±1.92	21.75±1.03
	Non pregnant	32.60±2.29	22.67±2.03	22.67±1.76	18.00±1.00
	P value	0.186^{NS}	0.568^{NS}	0.078^{NS}	0.059^{NS}

*(Note: **Significant at 1% level; NS = Non-significant; Different superscript different significantly from each others; SE = Standard error)*

The values of VER were observed in 20 each pubertal heifers and pluriparous cows on day 0th, 7th and 10th during the study period.

In Group I, the mean values of VER (ohm) on day 0, 7th and 10th were recorded as 35.10 ± 2.56, 34.10 ±3.67 and 29.80 ±2.05 ohms, respectively. In Group

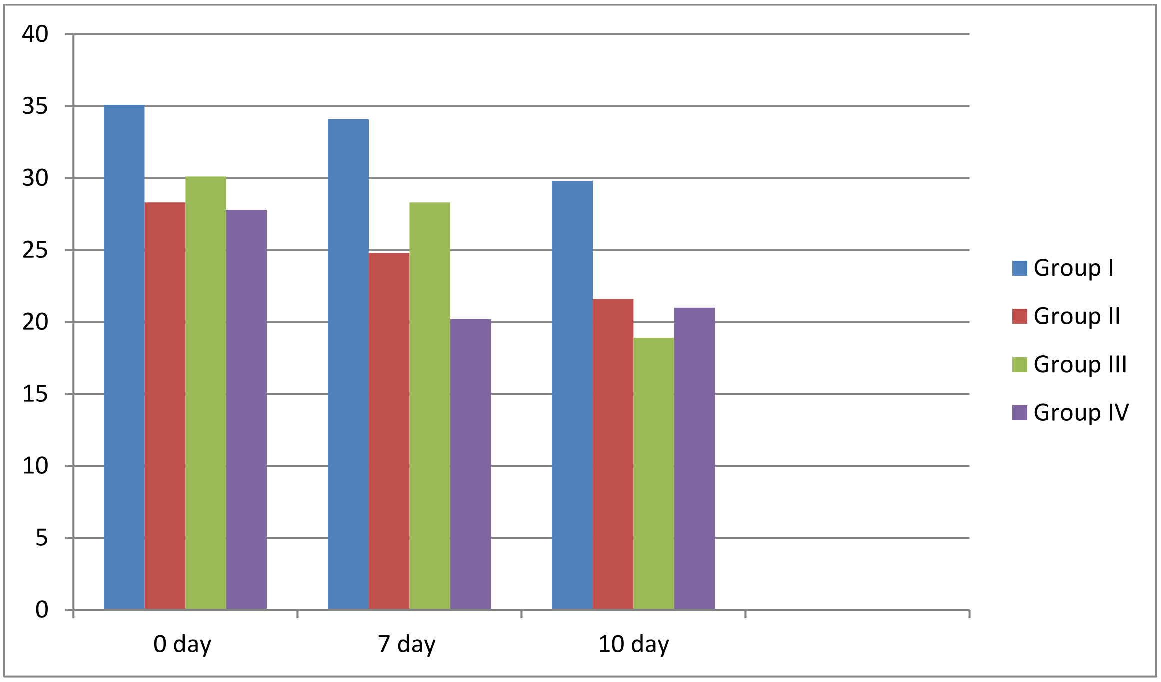

Fig 4.3 Average value of electronic heat detector

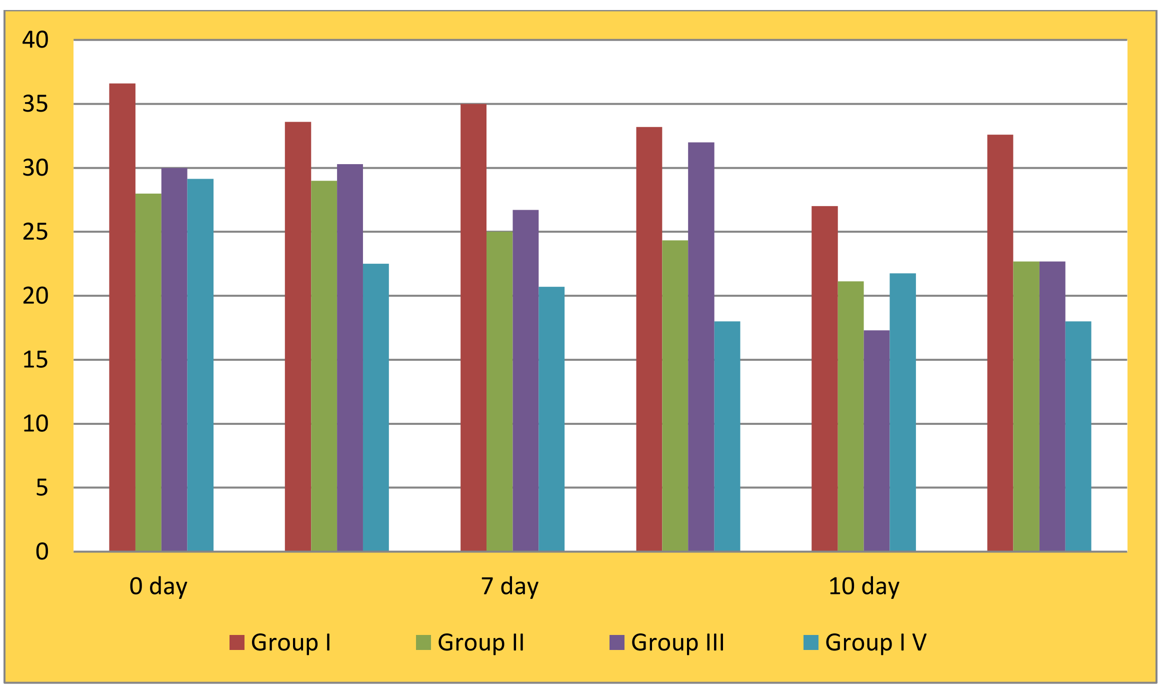

Fig 4.4 Value of electronic heat detector in pregnant and non pregnant animals

II, the mean values of VER on day 0, 7th and 10th were noted as 28.30±2.71, 24.80± 1.11 and 21.60± 1.10 ohms, respectively. In Group III, the mean values of VER on day 0, 7th and 10th were observed 30.10±1.33, 28.30±1.38 and 18.90±1.61 ohms, respectively. In Group IV, the mean values of VER on day 0, 7th and 10th were obtained 27.80±1.51, 20.20±1.82 and 21.00±0.96 ohms, respectively.

Mean values of VER were also recorded in pregnant and non pregnant animals from the respective groups on day 0, 7th and 10th. In Group I, mean values of VER in pregnant heifers were found 36.60±2.40, 35.00±6.10 and 27.00±3.13 ohm on day 0, 7th and 10th, respectively. Whereas mean values of VER in non pregnant heifers were 33.60±4.76, 33.20±4.81 and 32.60±2.29 ohm, ohm on day 0, 7th and 10th, respectively.

In Group II, mean values of VER in pregnant heifers were found 28.00±2.77, 25.00±1.46 and 21.14±1.39 ohm on day 0, 7th and 10th, respectively. Whereas mean values of VER in non pregnant heifers were found as 29.00±7.51, 24.33±1.86 and 22.67±2.03 ohm on day 0, 7th and 10th, respectively. Average mean value of VER in pregnant and non pregnant heifers was recorded as 31.58±2.22 and 31.88±3.85 ohm.

In Group III, mean values of VER in pregnant heifers were observed as 30.00±1.63, 26.71±0.97 and 17.29±1.92 ohm on day 0, 7th and 10th, respectively. Mean values of VER in non pregnant heifers in Group III were 30.33±2.85, 32.00±3.51 and 22.67±1.76 ohm on day 0, 7th and 10th, respectively.

In Group IV, mean values of VER in pregnant heifers were found 29.13±1.56, 20.75±1.96 and 21.75±1.03 ohm on day 0, 7th and 10th, respectively. Mean values of VER in non pregnant heifers were 22.50±0.50, 18.00±6.00 and 18.00±1.00 ohm on day 0, 7th and 10th, respectively. Average value of VER in pregnant and non pregnant cows was obtained as 29.53±1.09 and 27.20±2.48 ohm.

These values were indicated that VER was found to be higher during luteal phase while lower in follicular phase.

The average values of electronic heat detector (ohm) on 0th day was found to be non significant (p=0.078) amongst all groups. However, on 7th day, the average electronic heat detector values were found to be highly significant (p=0.001) across different groups. The highest (34.10 ±3.67 ohm) electronic heat detector values were found in Group I (Heifers) while the least values (20.20±1.82 ohm) were found in Group IV (Pluriparous cows). Further, on 10th day, average values of electronic heat detector were found to be highly significant (*p*=0.000) amongst all groups with highest (29.80 ±2.05 ohm) value in Group I while least (21.00±0.96 ohm) in Group IV (Table 4.7).

The comparison for average values of electronic heat detector was made between heifers and cows, it was observed that there was no significance (*p*=0.223) difference in the mean electronic heat detector values in heifers and cows (Table 4.8).

The decrease in the values of the electronic heat detector from day 0 to 10 among all groups indicates the importance of electronic heat detector values on day 10th for overall efficient heat detection.

The average values of electronic heat detector (ohm) on 0th day in pregnant and non pregnant animals was found to be non significant amongst all groups. However, on 7th day, the average electronic heat detector values in pregnant animals were found to be significant (*p*=0.013) across different groups whereas non significance difference was observed in non pregnant animals across different groups. Further, on 10th day, average values of electronic heat detector in pregnant animals were found to be significant (*p*=0.015) amongst all groups while highly significant (*p*=0.006) difference was observed in non pregnant animals (Table 4.9).

The comparison was made between value of electronic heat detector in pregnant and non pregnant animals in various groups with day 0, 7 and 10, respectively. On day 0, non significant difference in value of electronic heat detector in pregnant and non pregnant animal was observed across all groups except Group IV in which the difference was found to be highly significant (*p*=0.003). However, on day 7 and 10, the non significant difference in value of electronic heat detector in pregnant and non pregnant animals was observed across all groups (Table 4.10).

The similar results of VER values recorded by various authors like, Gupta and Mishra (1982) who examined the electrical resistance during estrus cycle in seven crossbred cows. The VER values were taken from one estrus to another. They observed that electrical resistances during estrus cycle vary considerably and lowest at the time of estrus and averaged 29.7 ohms. The electrical resistance thereafter was continued rising and the mean ranged between 38.5 to 50.6 ohms during day 4 to day 16 of estrus cycle. Thereafter the mean electrical resistance exhibited a decline with the approach of next estrus.

One more research findings are very closer to the observations of the present study. Abdel Rahim and Nazier (1987) who experimented in cows for detection of estrus by measuring electrical resistance of vaginal mucus membrane. The authors were reported the mean vaginal electrical resistance (VER) as 21± 1.2 ohms in estrus cows and 31±4.8 ohms in cows which was non estrus. Insemination on basis of such electrical resistance resulted in pregnancy rate was recorded as 94.00 %.

Rao and Rao (1982) observed vaginal electrical resistance of vaginal mucus at the time of insemination and correlated it with pregnancy rate in buffaloes. The authors were correlated between low resistance and higher pregnancy rate i.e. 66.60% and 60.00%, when artificial insemination was done at vaginal electrical resistance of 20-25 and 26-30 ohms, respectively.

Meena *et al.*, (2003) detected efficiency of vaginal electrical resistance measurement for estrus detection in cows also recorded mean vaginal electrical resistance during estrus, metestrus, diestrus, proestrus and anestrus as 32.68±0.46, 41.26±1.17, 50.23±0.55, 43.52±0.54 and 55.86±0.57 ohms, respectively.

Gupta and Purohit (2001) noted the changes in vaginal electrical resistance (VER) during different stages of estrus cycle and noticed that the mean VER during estrus, diestrus, proestrus and anestrus was 32.68 ± 0.46, 41.26 ±1.17, 50.23 ±0.55, 43.20 ±0.64 and 55.86 ± 0.57 ohms, respectively. They also reported insemination at lower VER distinctly improves pregnancy rate in buffaloes (81.40% vs 16.66%) with 26 and 40 ohms, respectively.

Aizinbudas *et al.* (1969) opined the vaginal electrical resistance of cervical mucus for cows in control was 435 ohms in diestrus and 261 ohms in estrus as against 547 and 352 ohms, respectively for cows given a diet lacking in Vitamin A.

Bohme and Buchholz (1977) observed that the electrical resistance in synchronized pubertal heifers and young cows was low on the day of estrus followed by follicular phase increase in the other phases of estrus cycle.

Leidl and Stolla (1976) studied a series of investigations on electric resistance measurements of the vaginal and cervical discharge during the estrus cycle and noted distinct relationship between the resistance measurements and the stages of estrus cycle in cows. Furthermore, the authors were mentioned lowest during estrus and in pathological condition of genital organs, such as follicular cysts and endometritis resulted in values corresponding to those observed during estrus.

4.4 Comparative conception rate (%) in pubertal heifers and pluriparous cows inseminated with sex sorted semen

The efficiency of insemination of dairy cows with sex sorted semen has remained an object of research to date. The aim of this study was to compare conception percentage with sex sorted semen in crossbred pubertal heifers and pluriparous cows.

In present study, Group I was included 10 crossbred pubertal heifers whereas Group III was included 10 crossbred pluriparous cows. Both groups were induced estrus with GPG+G protocol. 100.00 per cent animals were responded from both groups to the protocol. All animals were inseminated with sex sorted semen at fixed time artificial insemination. Three consecutive cycles were monitored and accordingly inseminations were performed. Per rectal examination was carried out after 60 days of insemination.

Conception rate by use of sex sorted semen in pubertal heifers and pluriparous cows were observed as in first estrus 3/10 (30.00%), second estrus 2/4 (50.00%) and remaining animals were not shown cyclicity in group I. In Group III, conception rate were noted for first estrus 4/10 (40.00 %), no animals shown second estrus 00 (00.00%) and third estrus 3/4 (75.00%). Overall conception rate was found as 5 / 10 (50.00%) and 7/10 (70.00 %) in pubertal heifers and pluriparous cows by use of sex sorted semen (Table 4.7& Fig 4.5).

As per above mentioned results, it can be concluded that high conception rate was found in pluriparous cows than the pubertal heifers by use sex sorted semen. These results might be due to low nutritional status, hormonal imbalance and managemental factor in heifers than the pluriparous cows. It needs to be pays attention on overall management in heifers during initiation of puberty.

Table 4.11 Overall conception rates (%) in pubertal heifers and pluriparous cows by use of sex sorted semen

Group	No. of animals inseminated	No. of animals pregnant	Conception rate
I	10	5	50.00 %
II	10	7	70.00 %

Similar reports were put on records by various authors viz. Karakaya-bilen *et al.*, (2019) who mentioned as 38.1 % and Sharma *et al.*, (2018) as 40.00 %. Furthermore, higher conception rates than the present study were recorded by Seidel and Schenk (2008), DeJarnette *et al*. (2009), Filhoa *et al*., (2010), Dawod and Elbaz (2020) and Maicas *et al*. (2019) as 47 %, 45 %, 51.8 %, 51.45 % and 45.5 %, respectively. The lower pregnancy rate of sex sorted semen than the present study was in harmony with the previous results of Doyle *et al*. (1999), Anderson *et al*. (2006), Norman *et al*. (2010), Healy *et al*. (2013), Karakaya *et al*. (2014) and Thomas *et al*. (2014a) reporting 25 %, 21 %, 17.2 %, 34.5 %, 31.6 %, 31.8 % and 36 % pregnancy rates, respectively.

The use of sex sorted semen for gender selection is today's need an advanced technology that has been widely and rapidly need to implement by dairy producers to increase the percentage of heifers born, capitalizing associated benefits and highlighting the importance to increase the milk production and reduce the burden of male calves on the farm. Reproductive capability plays an important role in the expected fertility rate with sex sorted semen. The present research results indicated that more technical management of crossbred cattle, proper handling of sperm, proper techniques of AI and use of a skilled inseminator is required for high success rate in terms of conception using sex sorted semen under field condition.

4.5 Comparative conception rate (%) in pubertal heifers and pluriparous cows inseminated with conventional semen

The aim of this study was to compare conception percentage with conventional semen in crossbred pubertal heifers and pluriparous cows. In present study, Group II which carried ten pubertal heifers and Group IV carried out ten pluriparous cows. These all animals were inseminated with conventional semen. Both groups were induced estrus with GPG+G protocol. All animals (100.00%) were responded for the mentioned protocol. All animals were inseminated with conventional semen at fixed time artificial insemination. Three consecutive cycles were monitored and accordingly inseminations were performed. Per rectal examination was carried out after 60 days of insemination for confirmation of pregnancy.

Conception rate by use of conventional semen in pubertal heifers and pluriparous cows were observed as in first estrus 5/10 (50.00%), second estrus 2/4 (50.00%) and one animal did not shown cyclicity in group II. Conception rate were noted for first estrus 2/10 (20.00 %), in second estrus conception was 6/7 (85.71%) and third estrus 00.00 (00.00%) in Group IV, respectively. Overall conception rate was found as 7 / 10 (70.00%) and 8/10 (80.00 %) in pubertal heifers and pluriparous cows by use of conventional semen. (Table 4.8 & Fig 4.6)

Table 4.12 Overall conception rates (%) in pubertal heifers and pluriparous cows by use of conventional semen

Group	No. of animals inseminated	No. of animals pregnant	Conception Rate
II	10	7	70.00%
IV	10	8	80.00%

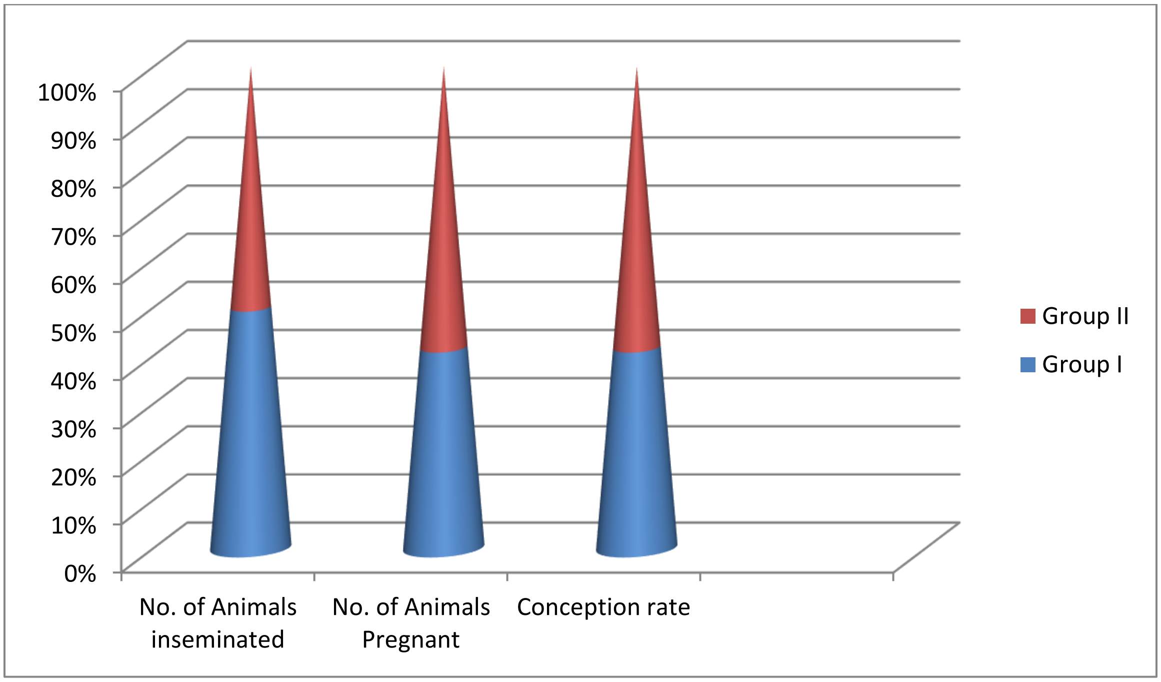

Fig 4.5 Overall conception rates (%) in pubertal heifers and pluriparous cows by using sex sorted semen

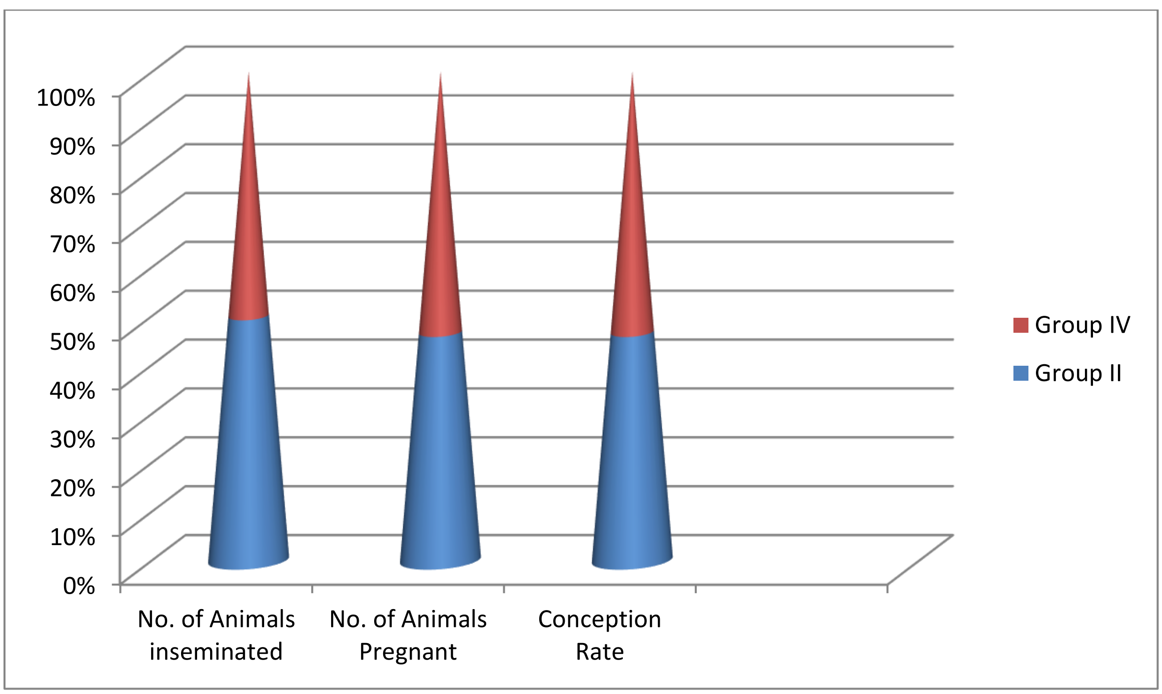

Fig 4.6 Overall conception rates in pubertal heifers and pluriparous cows by using conventional semen.

Similar reports were put on records by various authors viz. Silva *et al*.,(2015) who evaluated the effects of synchronizing estrus and ovulation to implement a timed artificial insemination (TAI) at first insemination on reproductive performance and cost per pregnancy in dairy heifers. Pregnancy at first AI did not differ between treatments (CON = 58.3 vs. TAI = 62.8%). Thomas *et al*., (2014b) achieved 54% conception, Lima *et al*,. (2011) got 61.4 % conception, Yilmaz *et al*., (2011) recorded pregnancy rates in cows and heifers as 57.50-60.00%. Xu and Burton (1999) observed the reproductive performance of heifers after estrus synchronization and fixed-time AI achieved 53.2% conceptions. Whereas some authors claimed lower conception rate in heifers as compared to the present research work like Pursley *et al*., (1995) 37.8%) and Pursley *et al.*, (1997) 35.1%.

In pluriparous cows, several reports concerned with higher pregnancy rates are reported. Bagal and Kadu (1989) who noted administration of 5 ml GnRH in postpartum crossbred cows and resulted as 100.00 % estrus response. Also the authors mentioned as 66.66 % conceived within 81 days requiring 1.16 services per conception. Twagiramungu *et al.*, (1992) who observed conception in postpartum beef cows and heifers 67.4 vs. 74.0%, Roy and Twagiramungu (1997) observed 61% conception rates during induced estrus in cows treated with GnRH–PGF2α–GnRH protocol. Geary *et al*., (1998) reported pregnancy rates of cyclic Ovsynch treated cows (59.00%). Keister *et al*., (1999) reported 70% pregnancy rate in cows following Ovsynch protocol. Vasconcelos *et al.,* (1999) reported pregnancy rate using conventional semen during AI as 61 % in animals after treatment. Sendag *et al*., (2001) synchronized anestrus and subestrus dairy cattle and heifer pregnancy rate in animals was 73.6%. Kesler, (2002) treated anestrus beef cows with CIDR and recorded as higher conception rate (57%). Baruselli *et al*., (2004) noted the pregnancy rate at induced estrus was 52% in cows treated with CIDR protocol. El-Zarkouny *et al.,* (2004) used CIDR with Ovsynch and reported pregnancy rates at 29 days (59.3% vs. 36.3%). Kasthuri, (2006) recorded over all conception rate in GnRH–PGF2α–GnRH treated postpartum anestrous crossbred cows as 70.00%. Ansari, (2007) achieved 60% first service conception rate and overall conception rate was found to be 90% in GnRH–PGF2α–GnRH treated cows.

Cevik *et al*., (2010) compared pregnancy rates between Heatsynch and CIDR-based synchronization protocol in dairy cows with conception rate 80.00%, and 53.33%, respectively which is similar to present research observations. Caraba *et al*., (2013) used Ovsynch protocol versus Cosynch protocol in Dairy Cows. Pregnancy per insemination was recorded at 60 days as of 57.00%. Dhami *et al.,* (2015) mentioned use of ovsynch protocol recorded as 100% estrus induction with overall conception rates at induced estrus of three cycles as 80%.

Some authors have shown lower conception rates than our study like Jeong *et al*., (2016) 34.4%. Khalil and Hussein (2019) reported conception rate was 36.64 ±1.32 using FTAI.

Among various studies on heifers and cows, overall conception rate was 75.00% (15/20) obtained by using conventional semen. Cows inseminated with sex sorted semen resulted overall conception rate was 60.00 % (12/20). Overall low conception rate was found by use of sex sorted semen than conventional semen in cattle.

The overall conception rate in artificially inseminated cows and heifers either with conventional or gender sorted semen in this study including all groups of cows was found to be 67.5 per cent % (27/40).

Data from this study indicated that the pluriparous cows had greater overall conception rate when inseminated with conventional semen than the cows inseminated with sex sorted semen during estrus synchronization.

4.6 Effects of estrus induction on cervico-vaginal discharge

Estrual discharge is considered a good indicator of reproductive health and potential fertility status of livestock. The present study was carried out to characterize the physical properties of cervical discharge collected while inseminating the cows and to relate with pregnancy. The estrual cervical discharge from experimental animals was collected before artificial insemination. The fern patterns i.e. typical, atypical and nil pattern was observed and recorded and also compared with the conception rates with pubertal heifers and pluriparous cows.

Results on conception rate in relation to the fern patterns of the cervical mucus are presented in table 4.13 & fig 4.7

Table 4.13 Conception rate (%) on the basis of cervico-vaginal mucus (CVM) in pubertal heifers and pluriparous cows

Animals	Fern pattern (%)			Conception rate
	Typical	Atypical	Nil	
Pubertal heifers n=20	60	40	00	60
Pluriparous cows n=20	75	25	00	75

The observations on fern pattern of cervico vaginal discharge in the present study were compared with the observations quoted by various researchers.

In the present study, the average percentage of typical, atypical and nil fern pattern of cervico-vaginal mucus in pubertal heifers induced with GPG=G protocol were recorded as 60.00, 40.00, 00.00 per cent, respectively whereas in pluriparous cows were 75.00, 25.00 and 00.00 per cent, respectively. The conception rate in estrus induced group of pubertal heifers and pluriparous cows was recorded as 60.00 and 75.00 per cent, respectively.

Percentage of typical and atypical fern patterns of estrual discharge were 87.50 % and 12.50 % in conceived cows reported by Bennur *et al.* (2004). The observations in the present study shown similar results of conception rates of typical (92 %) and atypical (00 %) in cervical mucus fern pattern during induced estrus in pubertal heifers and conception rates of typical (100 %) and atypical (20 %) in cervical mucus fern pattern during induced estrus in pluriparous cows. Dodamani *et al.* (2010) who reported the Deoni cows subsequently becoming pregnant exhibited considerably higher percentage of typical fern pattern (80.95 %) as compared to atypical fern pattern (19.05 %). The author was reported the cows failed to conceive had 56.6 % typical and 44.44 % atypical fern pattern. Ningwal *et al*. (2018) recorded the per cent incidence of typical fern pattern of cervicovaginal mucus in conceived crossbred cows as 86.67 % whereas in heifers as 83.33 %. Whereas, the frequency of atypical fern pattern of cervicovaginal mucus in conceived cows was 13.33 per cent and in heifers 16.66 per cent.

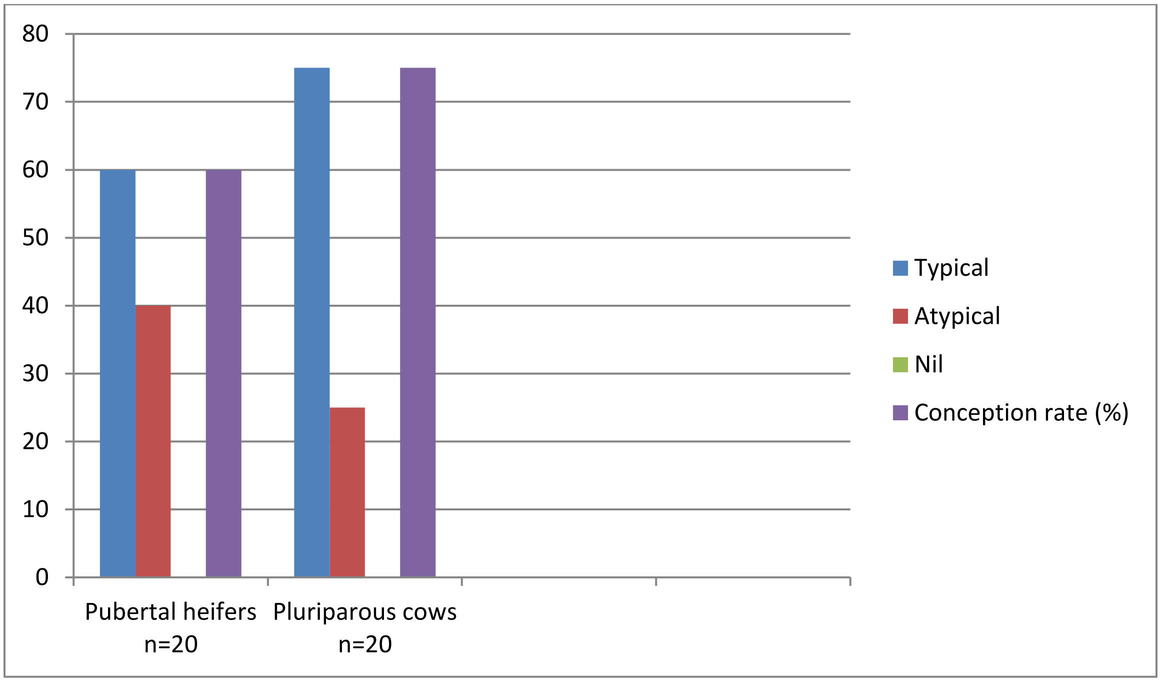

Fig 4.7 Conception rate (%) on the basis of cervico-vaginal mucus (CVM) in pubertal heifers and pluriparous cows

SUMMARY AND CONCLUSION

CHAPTER 5

SUMMARY AND CONCLUSIONS

The research work entitled **“Fertility Response of the Sex Sorted Semen in Crossbred Cattle”** was under taken on total 20 crossbred pubertal heifers and 20 pluriparous crossbred cows. The research work was carried on animals presented to Teaching Veterinary Clinical Complex, Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Udgir and Private Dairy Farms around Udgir Tahsil Dist Latur. Experimental animals were also selected from Dairy Farms in Tuljapur and Kalamb Tahsil Dist. Osmanabad. Cows were divided in four groups as Group I included 10 pubertal heifers assigned to be inseminated with sex sorted semen (2×10^6 cells per inseminating dose) and Group II carried out 10 pubertal heifers has received conventional semen (20×10^6 cells per inseminating dose). Group III and Group IV (n=10) was of pluriparous cows which received sex sorted semen and conventional semen, respectively. All selected animals were induced estrus by using GPG+G protocol and fixed time artificial insemination. The conception rate was calculated on the basis of total number of animals conceived divided by total number of animals inseminated. The pregnancy diagnosis was confirmed by per rectal examination after 60 days post insemination. A detailed study was carried out and conception rates in pubertal heifers as well as in pluriparous cows were compared in all groups.

It was observed that the duration of estrous across different groups was found to be non significant (p= 0.171). The average values of electronic heat detector (ohm) on 0^{th} day was found to be non significant (p=0.078) amongst all groups. However, on 7^{th} day, the average electronic heat detector values were found to be highly significant (p=0.001) across different groups. On 10^{th} day, average values of electronic heat detector were found to be highly significant (p=0.000) amongst all groups with highest (29.80 ±2.05 ohm) value in Group I while least (21.00±0.96 ohm) in Group IV.

Intensity of estrous was recorded as intense, intermediate and weak as 40, 50, 10; 40, 50, 10; 60, 40, 00 and 70, 30, 00 per cent in Group I, II, III and IV, respectively. Fern pattern were found to be typical and atypical as 50, 50; 70, 30; 70, 30 and 80, 20 per cent in Group I, II, III and IV, respectively.

Values of electronic heat detector was observed that there was no significance (p=0.223) difference in the mean electronic heat detector values in heifers and cows. The decrease in the values of the electronic heat detector from day 0 to 10 among all groups indicates the importance of electronic heat detector values on day 10th for overall efficient heat detection.

Conception rate (%) in induced estrus pubertal heifers inseminated with sex sorted semen and conventional Semen.

The heifers in groups I and II were subjected to estrus induction program consisting of administration of 5 ml (20 mcg) injection of Buserelin acetate **(**GnRH) by intra muscular route on day 0. On day 7, heifers were administered with 2 ml (250 mcg) injection of Cloprostenol (PGF2α) by intramuscular route. Second dose of Buserelin acetate **(**GnRH) with dose rate of 2.5ml (10mcg) was given on day 9, 48 h after PGF2α injection. Heifers were fixed time inseminated (FTAI) with sex sorted frozen semen or conventional frozen semen 24 h after second injection of Buserelin acetate **(**GnRH) analogue in subsequent groups. One more additional dose of GnRh at the rate of 2.5ml (10mcg) was given at the time of insemination. All heifers (100.00%) were shows estrus after receiving GPG+G protocol. The heifers were inseminated with sex sorted semen tented to have lower pregnancy rate than those inseminated with conventional semen following estrus synchronization program. The conception rate in heifers subjected sex sorted semen (group I; n=10) and conventional semen (group II; n=10) were recorded as 50.00 % and 70.00 %, respectively after induction of estrus with GPG+G protocol. The present study indicates overall conception rate including heifers of both the groups as 60.00 %.

Conception rate (%) in induced estrus pluriparous cows inseminated with sex sorted semen and conventional semen

Pluriparous cows in group III and IV were subjected to estrus induction program by using GPG+G. Cows were fixed time inseminated (FTAI) with sex sorted frozen semen or conventional frozen semen 24 h after second injection of Buserelin acetate **(**GnRH) analogue in subsequent groups. One more additional dose of GnRH at the rate of 2.5ml (10mcg) was given at the time of A.I. All pluriparous cows (100.00%) were responded for induction of estrus to GPG +G protocol. The conception in pluriparous cows was achieved 70.00 % and 80.00 % using sex sorted semen (group III) and conventional semen (group IV), respectively. Higher pregnancy rates were achieved with conventional semen than with sex sorted semen.

Comparative study of conception rate (%) between pubertal heifers and pluriparous cows with sex sorted semen.

As per findings in present research study, overall conception rate in heifers was 50.00 % obtained by use of sex sorted semen. Pluriparous Cows inseminated with sex sorted semen resulted in higher conception rate of 70.00 % than the heifers. The overall conception rate in artificially inseminated heifers and cows by using sex sorted semen was found to be 60.00 %. As per above mentioned results, it can be concluded that higher conception rate was found in pluriparous cows than the pubertal heifers by use sex sorted semen.

Comparative conception rate (%) in pubertal heifers and pluriparous cows inseminated with conventional semen

In present study, Group II which carried ten pubertal heifers and Group IV carried out ten pluriparous cows. All animals (100.00%) were responded for GPG+G protocol. All animals were inseminated with conventional semen at fixed time artificial insemination. Overall conception rate was found as 7 / 10 (70.00%) and 8/10 (80.00 %) in pubertal heifers and pluriparous cows respectively by use of conventional semen.

CONCLUSIONS

On the basis of the results recorded in the present study, it may be concluded that

1. Use of GPG + G protocol has achieved 100.00 per cent estrus induction response in anoestrus pubertal heifers and pluriparous cows.
2. Pluriparous cows exhibited higher percentage of intense estrous as compared to pubertal heifers.
3. It is concluded that higher conception rate can be achieved by insemination in correlation with typical fern pattern than atypical or nil pattern.
4. Vaginal electrical resistance values were recorded to be higher during luteal phase than the follicular phase.
5. It is concluded that sex sorted semen resulted in lower conception rate as compared to conventional semen in pubertal heifers and pluriparous cows.

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VITAE

The author Dr.Suresh Changdev Jadhav borned on 13 March, 1976 at Ruie, Tq. Bhoom and Dist Osmanabad of Maharashtra State. He completed primary schooling at Lonighat, Secondary School Certificate (SSC) examination in 1992 secured first class from Eknath Kolhe Vidya Mandir, Pachangri and subsequently completed higher secondary examination in 1994 from Yogeshwari Mahavidyalaya Ambajogai Dist. Beed secured first class.

Agriculturist family background and special attraction towards dairy animals has motivated him to take admission in veterinary profession. He has successfully completed B.V.Sc. & A.H. undergraduate degree course from College of Veterinary and Animal Sciences, Parbhani in Jan 1999. He actively participated in National Cadet Corps (NCC) unit of Marathwada Agricultural University, Parbhani and achieved ‘C’ certificate during his graduation studies and also participated in Animal Health work campaigns during undergraduate studies.

After completion of graduation, he has served as field veterinarian in various renowned dairy sector organizations in western Maharashtra like Amrutsagar Co operative Milk Union, Akole, Dist. Ahmadnagar, Rajarambapu Patil Milk Union, Islampur, Dist. Sangali, Sahyadri Agro Ltd., Baramati Dist. Pune. He has also worked as Assistant Project Manager in one of the most well-known NGO, JK TRUST Gram Vikas Yojna, Thane which is working in Artificial Insemination and IVF Technology. Presently, he is working with Govt. of Maharashtra in Zillha Parishad, Osmanabad.

Due to his desire to have higher studies he joined postgraduate (M.V.Sc.) studies in the discipline of Animal Reproduction, Gynaecology and Obstetrics in the Department of Animal Reproduction, Gynaecology & Obstetrics, College of Veterinary and Animal Sciences, MAFSU, Udgir.

He has attended number of National and International seminars on different topics which were held at different places in India. He has also received certificates for successfully attending technical trainings arranged by MAFSU and other veterinary universities of India. He is having keen interest in clinical practice and has worked sincerely during post-graduate studies at Department of ARGO, TVCC, COVAS, Udgir. He has published four popular articles in Marathi daily agricultural news paper Agrowon.

Thesis Abstract

THESIS ABSTRACT

a)	**Title of Thesis**	:	**FERTILITY RESPONSE OF THE SEX SORTED SEMEN IN CROSSBRED CATTLE**
b)	**Full Name of Student**	:	**MR. JADHAV SURESH CHANGDEV**
c)	**Name & Address of Major Advisor**	:	**Dr. A. D. Patil** Assistant Professor, Dept. of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Udgir, Dist. Latur (M.S.)
d)	**Degree to be awarded**	:	M.V.Sc.
e)	**Year of award of degree**	:	2021
f)	**Major Subject**	:	Animal Reproduction, Gynaecology and Obstetrics
g)	**Total No. of pages in thesis**	:	96
h)	**Number of words in abstract**	:	**355**
i)	**Signature of the student**	:	
j)	**Signature, Name and address of forwarding authority**	:	**(Dr. A. D. Patil)** **Assistant Professor and Head** **Department of Animal Reproduction,** **Gynaecology and Obstetrics.** **COVAS, Udgir**

ABSTRACT

The present research work entitled **"Fertility response of the sex sorted semen in crossbred cattle''** was carried out in the Department of Animal Reproduction, Gynaecology and Obstetrics, COVAS, Udgir. A total 20 crossbred heifers and 20 pluriparous cows were selected for the present study. The GPG+G protocol was used as Inj. GnRH @20 mcg on day 0, inj. PGF2α @2 ml on day 7, inj. GnRH@ 10mcg administered on day 9 and inj. GnRH@10 mcg at the time of

A.I. on day 10, FTAI was performed 24 h after the second GnRH injection. Same protocol was used to all groups for induction of estrus.

Animals were divided into four equal groups. Group I (n=10) was included pubertal heifers and inseminated with sex sorted semen. Group II (n=10) was carried out pubertal heifers and inseminated with conventional semen, Group III (n=10) was induced pluriparous cows and inseminated sex sorted semen and Group IV (n=10) used conventional semen in pluriparous cows.

Duration of estrus was observed as 25.2±4.54, 24.60±2.27, 20.2±1.87, 30.00±2.68 hrs in Group I, II, III and IV, respectively. Estrus induction response was found as 100.00%, Intensity of estrus (%) as intense (40, 40, 60, 70), intermediate (50,50,40,30) and weak (10,10,0,00,) among all four groups. Fern pattern were found to be typical and atypical as 50, 50; 70, 30; 70, 30 and 80, 20 per cent in Group I, II, III and IV, respectively. Values of electronic heat detector was observed that there was no significance (p=0.223) difference in the mean electronic heat detector values in heifers and cows. The decrease in the values of the electronic heat detector from day 0 to 10 among all groups indicates the importance of electronic heat detector values on day 10th for overall efficient heat detection.

The overall conceptions achieved in present research by use of sex sorted semen and conventional semen was 50.00, 70.00, 70.00 and 80.00 per cent in pubertal heifers and pluriparous cows, respectively.

It is concluded that sex sorted semen resulted in lower conception rate as compared to conventional semen in pubertal heifers and pluriparous cows.

Keywords: sex sorted semen, conventional semen, GPG+G protocol, cattle

प्रबंध

सारांश

प्रबंध सारांश		
अ. प्रबंधाचे शिर्षक	:	**संकरीत गाई मध्ये लिंगविनिश्चीत वीर्यमात्रेचा प्रजनन प्रतिसाद**
ब. विद्यार्थ्यांचे पूर्ण नाव	:	**श्री जाधव सुरेश चांगदेव**
क. मार्गदर्शकाचे नाव व पत्ता	:	**डॉ. अनिल दि. पाटील** सहाय्यक प्राध्यापक पशुप्रजनन, मादीरोग, व प्रसूतिशास्त्र विभाग पशुवैद्यकिय महाविद्यालय, उदगीर, जि.लातूर ४१३५१७
ड. प्रदान केली जाणारी पदवी	:	एम.व्ही.एस.सी.
इ. पदवी प्रदान करण्याचे वर्ष	:	२०२१
फ. मुख्य विषय	:	पशुप्रजनन, मादीरोग व प्रसूतिशास्त्र
ह. प्रबंधाची एकूण पृष्ठे	:	९४
ळ. प्रबंध सरांशातील एकूण शब्द	:	३२१
क्ष. विद्यार्थ्यांची स्वाक्षरी	:	
ज्ञ. प्रबंध कार्यवाहीस्तव पाठविणाऱ्या अधिकाऱ्यांची स्वाक्षरी, नाव व पत्ता	:	**डॉ. अनिल दि. पाटील** सहाय्यक प्राध्यापक व विभागप्रमुख पशुप्रजनन, मादीरोग, व प्रसूतिशास्त्र विभाग पशुवैद्यकिय महाविद्यालय, उदगीर, जि.लातूर ४१३५१७

सारांश

प्रस्तुत संशोधन प्रबंधाचे शिर्षक, 'संकरीत गाई मध्ये लिंगविनिश्चीत वीर्यमात्रेचा प्रजनन प्रतिसाद"' असे असून संबंधित संशोधन पशुप्रजनन, मादीरोग व प्रसूतिशास्त्र विभाग, पशुवैद्यकिय महाविद्यालय, उदगीर, जि. लातूर येथे करण्यात आला.

या संशोधन कार्यामध्ये २० संकरीत कालवडी व २० संकरीत गायींचा समावेश करण्यात आला. एकावेळी सर्व जनावरे माजावर आणण्यासाठी शून्य व्या दिवशी इंजेक्शन जी.एन.आर.एच. @ २० एमसीजी मासामध्ये देण्यात आली. ७ व्या दिवशी पी.जी.एफ.टू. अल्फा इंजेक्शन २ मि.लि. मासामध्ये देण्यात आले. त्यानंतर ४८ तासांनी इंजेक्शन जी.एन.आर.एच. दुसरी @ १० एमसीजी व एक अतिरिक्त जी.एन.आर.एच @ १० एमसीजी रेतानावेळी देण्यात आली. कालनिश्चित कृत्रिम रेतन जी.एन.आर.एच च्या दुसऱ्या मात्रे नंतर २४ तासांनी करण्यात आले. २० संकरीत कालवडी व २० गाई चार समान गटामध्ये विभागण्यात आल्या. गट १ मध्ये १० प्रजननक्षम कालवडी ना लिंगविनिश्चीत रेत मात्रा वापरून रेतन करण्यात आले. गट २ मध्ये १० प्रजननक्षम कालवडीना पारंपारिक रेत मात्रा देऊन कृत्रिम रेतन करण्यात आले. गट ३ मध्ये १० संकरीत गाईचा समावेश करण्यात आला व त्यांना लिंगविनिश्चीत रेत मात्रा वापरून रेतन करण्यात आले. गट ४ मध्ये १० संकरीत गाई निवडण्यात आल्या, त्यांना पारंपारिक रेत मात्रांचा वापर करून रेतन करण्यात आले.

माजाचा कालावधी अनुक्रमे गट १,२,३, व ४ मध्ये सरासरी २५.२±४.५४, २४.६±२.५७, २०.२±१.८७, ३०.००±२.६८ तास राहिला. एकावेळी माजावर आणण्यासाठी जी कार्यपद्धती वापरण्यात आली त्यामध्ये १००.००% जनावरे माजावर आले. सर्व गटामध्ये माजाची तीव्रता (%) अतितीव्र (४०,४०,६०,७०), मध्यम (५०,५०,४०,३०) व क्षीण (१०,१०,०,०) अशी

नोंदविण्यात आली. बळसाचे फर्ण पानासारखे नमुने अभ्यासण्यात आले. यामध्ये गट १,२,३,व ४ मध्ये ठराविक व वैशिष्ठ्यपूर्ण नमुने अनुक्रमे ५०,५०;७०,३०;७०,३० व ८०,२० असे राहिले. गाई व कालवडीमधील इलेक्ट्रोनिक माज तपासणी यंत्राच्या सरासरी नोंदीमधील फरक गैर महत्वपूर्ण (पी=०.२२३) आला. ० ते १० दिवसामधील इलेक्ट्रोनिक माज तपासणी यंत्राच्या सर्व गटाच्या सरासरी नोंदीमधील उतरणं माज तपासणी यंत्राची सक्षमता दर्शवते.

सर्वसाधारणपणे प्रस्तुत संशोधन कार्यामध्ये लिंग विनिश्चीत व पारंपारिक रेत मात्रा वापरून १ ते ४ गटामध्ये अनुक्रमे ५०, ७०, ७० व ८० टक्के गर्भधारणा झालेली आहे.

तसेच सबंधित संशोधना आधारे एक निष्कर्ष काढता येतो की वयात आलेल्या कालवडी तसेच गाई मध्ये लिंग विनिश्चीत रेत मात्रांचा गर्भधारणा दर हा पारंपारिक रेत मात्रांपेक्षा कमी आहे.