

**FERTILITY OF SEXED SEMEN IN OVSYNCH AND
DOUBLE PG OVSYNCH PROTOCOLS IN BUFFALOES**

T H E S I S

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In partial fulfillment of requirements for the Degree of

**MASTER OF VETERINARY SCIENCE
IN**

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BY

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I hereby declare that the experimental research work and interpretation of the thesis entitled “**FERTILITY OF SEXED SEMEN IN OVSYNCH AND DOUBLE PG OVSYNCH PROTOCOLS IN BUFFALOES**” 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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Date :

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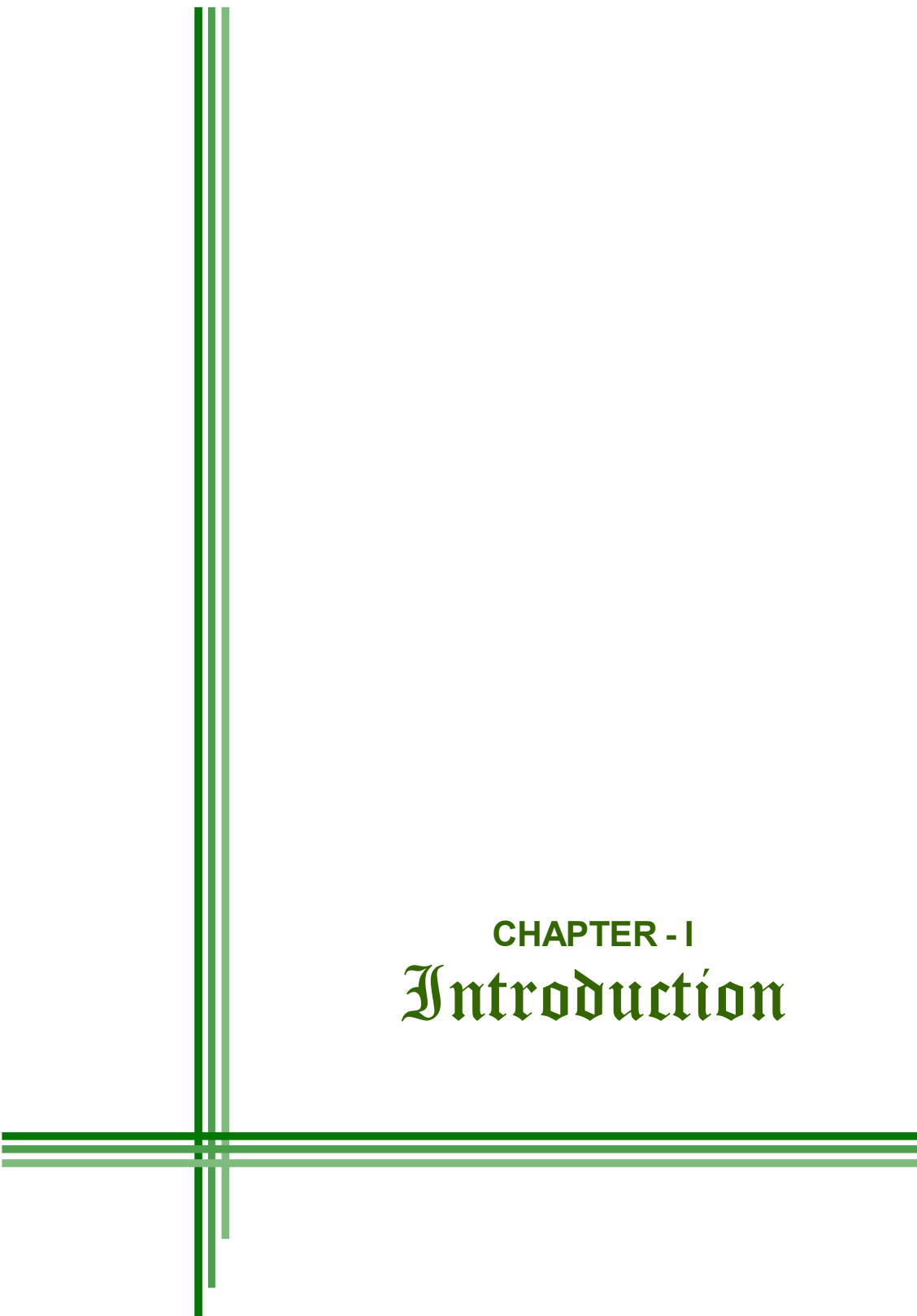
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LIST OF ABBREVIATIONS

Abbreviation/Symbols	Full form
%	: Percent
/	: Per
@	: At the rate
+	: Plus
<	: Less than
=	: is equal to
>	: Greater than
±	: more than less than
≤	: Less than or equal
≥	: More than or equal
AI	: Artificial Insemination
Avg.	: Average
°F	: Degree Fahrenheit
µg/kg	: Microgram per kilogram
µl	: Microliter
CL	: Corpus Luteum
CD	: Critical difference
cm/s	: Centimeter per second
CIDR	: Controlled internal drug releasing intravaginal device
CRD	: Completely randomized design
CR	: Conception Rate
CV	: Coefficient of variation
eCG	: Equine chorionic gonadotropin
ED and AI	: Estrus Detection and Artificial Insemination
<i>et al.</i>	: And others/ And Association
<i>etc.</i>	: et cetera/And so forth
GDP :	Gross domestic product
Fig.	: Figure
FSH	: Follicle stimulating hormone
FTAI	: Fixed Time Artificial Insemination
GnRH	: Gonadotropin releasing hormone
hCG	: Human Chorionic Gonadotropin
HF	: Holstein Frisian
hr/hrs	: Hour/hours
<i>i.e.</i>	: that is
I/V	: Intravenous
I/M	: Intramuscular
Inj.	: Injection
IU	: International units
IU/kg	: International units per kilogram
IV	: Intravenous

kg	:	Kilogram
LH	:	Luteinizing hormone
Ltd	:	Limited
mg	:	Milligram
ml	:	Milliliter
mm	:	Millimeter
n	:	Number of animals
ng/ml	:	Nanogram/ milliliter
nmol/L	:	Nano-moles per liter
NS	:	Non-significant
P4	:	Progesterone value
PGF ₂ α (PG)	:	Prostaglandin F ₂ alpha
pH	:	Power of hydrogen ion
PPC	:	plasma progesterone concentration
PR	:	Pregnancy Rate
Pvt	:	Private
SC	:	Subcutaneous
SE	:	Standard error
SEM	:	Standard error of mean
TAI	:	Timed Artificial Insemination
vs	:	Versus
X	:	Magnification
α	:	Alpha



CHAPTER - I
Introduction

CHAPTER - I

INTRODUCTION

The domestic water buffalo (*Bubalus bubalis*) contributes a significant share of global milk production and is the major milk producing animal in several countries. The river and swamp buffalo are the two subspecies of water buffalo. River buffaloes constitute approximately 70 percent of the world water buffalo population and accounts for a substantial share of total milk production in India and Pakistan. Swamp buffaloes are smaller and have lower milk yields than river buffaloes. They are present mainly in Eastern Asia and are primarily raised for draught power (FAO, 2021).

India continues highest milk producing county globally with 198.40 million tonnes. In India, as per 20th livestock census- 2019 the buffalo population is 109.85 million and nearly 49 % of the milk production is contributed by Indigenous and non descript buffaloes in 2019-20 (Annual Report, DAHD-2020-21). Buffalo named as “black gold” and plays a pivotal role in economical development through contributions to milk, meat, hides and draft power for agricultural operations. Buffalo forms part of the property, possession, profession of rural farmers as well as are an easily ‘convertible currency’ and a reliable ‘living bank’ to serve the immediate needs of the rural masses in several communities (Nanda *et al.*, 2003).

The main constrain in commercial buffalo milk production is low reproductive efficiency which mainly include animals late age at first calving, the seasonality of estrus, and the long calving interval and dry period remains a major economic problem globally and its incidence is higher in India. The post partum anestrus, weak or silent estrus, lower conception rate through artificial insemination and more number of services per conception are major reproductive problems encountered in India (Ingawale and Dhoble, 2004). This affects not only total milk production of buffalo but also calf crop in her reproductive life affecting profitability of herd.

Maintaining optimum inter calving period of 14-15 months in buffaloes by adopting estrus synchronization with timed artificial insemination with sexed semen technology is most prominent reproductive tool at present for

enhancing milk production, productivity and more number of replacing high merit heifers in commercial buffalo herd.

Ovsynch is one of the most adopted estrus synchronization protocol consisting of two injections of gonadotropic releasing hormone (GnRH) combined with single administration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) used for synchronization of ovulation and fixed time artificial insemination (Singh, 2014). The advantage of Ovsynch protocol is estrus synchronization can be done in buffaloes at all the stages of cycle (Paul and Prakash, 2005). Synchronization of estrus as well ovulation occurs in Ovsynch protocol is found very effective for improving reproductive performance in buffaloes (Paul and Prakash, 2005; Ghuman *et al.*, 2009) in Murrah buffalo heifers (Roy and Prakash, 2009) as well as during non breeding season (Karen and Darwish, 2010).

The major limitation of Ovsynch protocol is lack of luteolysis after the $PGF_{2\alpha}$ injection and also inability of GnRH to turnover dominant follicles late in the estrus cycle leading to premature estrus. Animals that ovulate to the first GnRH treatment generally have greater fertility than cows that do not ovulate (Keskin *et al.*, 2010). However, the new CL, induced by the first GnRH treatment in Ovsynch, is inappropriately regressed with a single treatment with prostaglandin $F_{2\alpha}$. Lack of complete regression of the CL to the $PGF_{2\alpha}$ treatment has been observed in 10 to 25% of cows treated with Ovsynch and small elevations in circulating P4 near AI, due to inadequate CL regression, have reduced fertility (Brusveen *et al.*, 2009; Martins *et al.*, 2011; Wiltbank and Pursley, 2014). In Ovsynch protocol, lack of regression of the CL is an important problem, leading to reductions in fertility during Ovsynch or modifications of Ovsynch protocols (Martins *et al.*, 2011; Giordano *et al.*, 2012; Wiltbank *et al.*, 2014). The incomplete CL regression is more likely in cows with a new CL than in cows that did not ovulate to the first GnRH treatment (Giordano *et al.*, 2012). To overcome the problem with inadequate CL regression during timed AI protocols, two general strategies have been used. One strategy is to give an increased dose of PGF during either the 5 or 7d Ovsynch protocols (Ribeiro *et al.*, 2012; Giordano *et al.*, 2013). A second strategy is to give a second dose of PGF on the subsequent day after the first

PGF treatment of a 5 or 7d Ovsynch protocol. In the 7-d protocol, increasing the dose of cloprostenol from 500 to 750 µg increased CL regression in multiparous but not primiparous cows and tended to increase fertility (Giordano *et al.*, 2013). An increased percentage of cows with complete CL regression (<0.4 ng/mL 56 h after PGF) was found after two (95.6%) compared with single (84.6%) PGF₂α treatment (Brusveen *et al.*, 2009). Carvalho *et al.*, (2015) respond that a double PGF₂α injection during the Ovsynch protocol was able to boost luteolysis and reported cows treated with double PGF₂α injections and with lower P4 concentration on the day of TAI had better reproductive outcomes.

Cervical mucus is a visco-elastic secretion of constantly secreting mucus producing cells of endo-cervix (Glover, 1960) and acts as a mechanical barrier to prevent intruding organisms. It is a complex biomaterial having a vital protective function in the cervix during pregnancy. Bovine cervical mucus exists into two phases, aqueous and gel (Rutllant *et al.* 2005). The aqueous phase contains mostly water (92-95%) with some ion and metabolites (Tsiligianni *et al.*, 2001) whereas; the glycoproteins (mucins) are the major component of the gel phase (Pluta *et al.*, 2011). Colour, appearance, pH and electrical conductivity along with other rheological properties like spin barkeit value, elasticity, viscosity and fern (arboisation) pattern are the most important properties in relation to fertility (Pandey *et al.*, 1983). These properties vary with endocrinological status of reproductive cycle and directly associated with estrogen: progesterone ratio and fertility status of dairy animals (Rangnekar *et al.*, 2002). The rheological properties of cervical mucus becomes more plentiful, watery and less viscous during follicular phase, which facilitates the transport of spermatozoa in female reproductive tract. However, it becomes opaque, thick, viscous and scanty during luteal phase not favorable for sperm migration (Rutllant *et al.*, 2005). Cervical secretion becomes more copious and watery during estrogen dominating phase of ovarian cycle while viscosity of mucus increases under high progesterone concentration. The occurrence of crystallization is common to all types of mucus. But degree of crystallization or arboisation pattern in cervical mucus is under the control of two ovarian hormones *viz.* estrogen and progesterone. The phenomenon of crystallization in cervical mucus progresses under the influence of estrogen

whereas progesterone diminishes the formation of arbonisation pattern (Tsiligianni *et al.*, 2001). Thus it can be quite useful in predicting the onset of estrus, different stages of estrus and ovulation time in cattle (Alena *et al.*, 2008).

Artificial insemination is proven technology for enhancing milk production. At present AI coverage is upto 30 % breedable population of bovine in India and in 2019-20, 80 million AI were performed in India with average conception rate of 35 % (Annual Report, DAHD-2020-21). The predetermining the sex through application of sexed semen has valuable impact on livestock industry because of its economic gains. Sex of calf is an important aspect of animal husbandry and buffalo farmers prefer female calves as progeny for milk production. The projected demand of milk by 2030 is estimated to be 266.5 million MT. To meet the increasing demands, it is necessary to substantially increase the number of elite buffalo females which can achieved by utilization of sexed semen in buffaloes. Sawant (2021) reported 40 % conception rate with sexed semen when used in natural spontaneous estrus in buffaloes. For the larger practical application of sexed semen in buffalo breeding it would be great interest when used in synchronized estrus with its effect on fertility and in economic terms. The studies on practical applicability of sexed semen are very meager in estrus synchronized buffaloes at field level. Considering this research gap, the effect of second dose of PGF₂ α in Ovsynch protocol on fertility as well as sexed semen in Ovsynch and double PG Ovsynch protocols in buffaloes, the present research work was planned with following objectives

Objectives

1. To study the efficacy of Ovsynch and double PG Ovsynch protocols for estrus synchronization in buffaloes
2. To record the estrus signs, pH and fern pattern in Ovsynch and double PG Ovsynch protocols in buffaloes
3. To study the pregnancy rate of sexed semen in Ovsynch and double PG Ovsynch protocols in buffaloes



CHAPTER - II
Review of Literature

CHAPTER – II

REVIEW OF LITERATURE

In cattle it took about 20 years from establishing the principle for preselecting sperm according to their DNA content, till the first commercialized sex sorting sperm use in artificial insemination. Thousands of calves have now been born from sperm sorted for sex chromosomes by flow cytometry / cell sorting based on DNA content. The majority have been produced in the United States, United Kingdom, and Argentina, with less numbers in other countries. This technology was in various phases of commercialization for cows in other countries (Garner and Seidel, 2003).

In India, Paschim Banga Go-Sampad Bikash Sanstha (PBG SBS), a Government of West Bengal organization, initiated sorting of semen using high speed semen sorter or flow cytometer (Influx, Becton Dickinson, Biosciences, San Jose, CA, USA) installed on 15th August, 2009 under RKVY with a total outlay of Rs. 2.90 crores, during 2007 – 08 and 2008 – 09 and completed in November, 2009 at Frozen Semen Bull Station, Haringhata. They reported first male calf named Shreyas, born on 1st Jan 2011 using sexed semen. Later female calves were also successfully born to sexed semen. The conception rates observed were 20.7% in cows and 35.3% in heifers using sexed semen. The purity of X sorted semen was found to be higher compared to Y-sorted semen (Biswas *et al.*, 2013). Kerala also reported birth of two sexed semen calves to Jersey crossbred heifers and Holstein Friesian crossbred cows respectively at Vakkavu in Nenmara, Palakkad under a pilot project jointly taken by Kerala Livestock Development Board (KLDB) and Department of Animal Husbandry.

In India presently sexed semen laboratories are functional out of which two are located in Maharashtra. In the present chapter, review the pertaining to estrus synchronization with Ovsynch and double PG Ovsynch protocol, cervical mucus properties and pregnancy rate of sex sorted and conventional semen was reviewed.

2.1. Synchronization of Estrus with Ovsynch Protocol

Baruselli *et al.* (2001) synchronized two hundreds seventy five buffaloes with two gonadotrophin releasing hormone agonist. In Group-I (n=132) buffaloes were treated with 20 ug Buserlin on day 0, 15 mg of PGF₂ α , 7 day later and 10 ug Buserlin on the day 9. In Group-II (n=138) buffaloes were treated with 50 ug of Leceirelin on day 0, 15 mg of PGF₂ α on day 7 and 25 ug of Leceirelin on day 9. A.I. was performed 16 h after the last GnRH injection. The conception rate was 47 percent (62/132) and 50 percent (69/138) in Group-I and II, respectively.

Berber *et al.* (2002) evaluated ovulation synchronization protocol in buffaloes. Total three hundred five buffaloes were selected and divided in Group-I (n=154), received 20 ug GnRH on day 0 (Buserlin), 15 mg PGF₂ α (Luprositol) on day 7 and 10 ug GnRH on day 9 whereas in Group-II (n=151), received 20 ug GnRH on day 0, 15 mg PGF₂ α on day 7 and 12.5 mg Luprotial-lutropin-v on day 9. The conception rate was 56.5 percent and 64.2 percent in Group I and II, respectively.

Baruselli *et al.* (2003) treated one thousand fifty three buffaloes in random days of postpartum periods (> 40 day) with 20 mg of GnRH on day 0, 15 mg of PGF₂ α on day 7 and 10 mg of GnRH on day 9. A. I. was performed 16 h after the last GnRH injection without estrus detection. The conception rate was 45.4 percent.

Neglia *et al.* (2003) synchronized one hundred eleven Italian Mediterranean buffalo cows with 100 ug GnRH on day 0, 375 ug PGF₂ α on day 7 and 100 ug GnRH on day 9 (Ovsynch). Buffaloes were inseminated twice 18 and 42 h after the second injection of GnRH. The pregnancy rate was 36.0 per cent.

Berber *et al.* (2005) selected eighty seven multiparous Murrah buffaloes with postpartum period > 40 days. The selected buffaloes were divided into two experimental groups. All the buffaloes were treated with 25mg of GnRH injection on (d 0) (Leceirelingestraanphus) and 150 mg PGF₂ α injection (d- cloprostenol) on d 7. In Group-II, forty three buffaloes received 1

mg estradiol benzoate (estrogen) on day 8 where as in Group-I, forty four buffaloes received 25 mg GnRH injection on d 9. All the buffaloes were inseminated 30 h after estradiol benzoate (Group-II) or 16 h after second GnRH injection (Group-I). The conception rate was 47.7 percent (21/44) and 34.90 percent (15/43) in Group-I and II, respectively.

De-Rensis *et al.* (2005) treated sixty two cyclic pluriparous buffaloes with Ovsynch protocol. All the buffaloes were inseminated 16 to 20 h after the second GnRH administration. The overall conception rate was 43.7 per cent.

Paul and Prakash (2005) conducted two experiments to assess the timing, synchrony of ovulation and pregnancy rate in Murrah buffaloes (*Bubalus bubalis*) treated with the Ovsynch (GnRH- PGF₂ α - GnRH) protocol. In first experiment, ten non lactating cycling buffaloes were treated with 10 μ g of GnRH analogue i. m. (Buserelin acetate) without regard to the stage of estrus cycle, followed by 25 mg of PGF₂ α i.m. (Dinoprost tromethamine) 7 days later while second treatment of same GnRH analogue (10 μ g i.m.) was given 48 h after PGF₂ α treatment. Ovulation was confirmed by transrectal palpation (at 2 h intervals) from the second GnRH treatment to detection of ovulation or up to 96 h after the second GnRH treatment. Ovulation occurred in nine buffalo (90 percent), 23.3 \pm 1.3 h after the second GnRH treatment. In experiment two, fifteen lactating cycling buffaloes were treated by Ovsynch protocol, with fixed time AI, 12 and 24 h after second GnRH treatment and seventy five lactating buffaloes were inseminated, approximately 12 h after detection of spontaneous estrus. Pregnancy rate was 33.30 percent for TAI and 30.70 percent for buffaloes inseminated following spontaneous estrus.

Bhosrekar (2006) synchronized four hundred graded Murrah buffalo suffered from post partum anestrus (200 to 250 days) from Guntur district of Andhra Pradesh. The buffaloes were given deworming, all vaccinations before synchronization and treated with 2.5 ml Receptal, 5 ml Illirin and again 2.5 ml of Receptal intramuscularly on 0, 7 and 9 day intervals, respectively. Two inseminations were carried on 16 h and 24 h after last GnRH injection. Low pregnancy rate was observed to first AI whereas, 77.70 percent

conception rate was observed after overall three subsequent inseminations after synchronized estrus as compared to 17.5 per cent in control buffaloes.

Ali and Fahmy (2007) selected eighteen buffalo cows and divided in cyclic (n=10) and noncyclical (n=8). All the buffalo cows were treated with GnRH on the day 0, PGF₂α on day 7 and second dose of GnRH on the day 9, whereas A.I. was carried out 14h later. Out of treated buffalo cows, 80 and 87.5 percent cyclic and noncyclical buffalo cows responded for luteolytic treatment. Following second GnRH, ovulation occurred in 80 percent cyclic cows whereas 100 percent noncyclical cows. Conception rate was 60 percent and 37.5 percent in cyclic and noncyclical buffalo cows, respectively.

Carvalho *et al.* (2007) synchronized thirty six buffaloes with 25 ug of GnRH (Leceirelin) i/m on day 0 and 150 ug of PGF₂α (d- Cloprostenol; prolise; arsa; Argentina) on day 7 while on day 9 buffaloes received 25 ug of GnRH i/m. All the buffaloes were inseminated, 16 h after the last hormone treatment. Out of 36 buffaloes, 20 buffaloes (61.1 percent) were conceived.

Ingawale *et al.* (2007) synchronized fifteen post partum anestrus buffaloes with 90 to 150 day of postpartum anestrus. All the buffaloes were injected with Receptal 5 ml (day 0), injection Illerin 5 ml (day 7) and injection Receptal 5 ml (day 9), intramuscularly. Estrus was noted and timed inseminations were carried out 24 h after second dose of Receptal (day 9). Out of 15, 13 (86.60 percent) buffaloes exhibited estrus whereas 3 (23.07) percent buffaloes remain pregnant.

Rohilla *et al.* (2007) studied the effect of pre-treatment ovarian status on fertility of Ovsynch treated summer anestrus postpartum (64-180 day, 1-3 lactation) Murrah buffaloes (n=12). Based on ovarian status on day 0, buffaloes were classified into Category- I (presence of corpus luteum), Category-II (presence of at least one large follicle > 9 mm) and Category-III (no large follicle or corpus luteum present). Buffaloes of all category received Ovsynch treatment consisting of GnRH (Buserlin acetate, 10 ug), PGF₂α (Tiaprost, 750 mg) and GnRH (Buserlin acetate) on day 0, 7 and 9, respectively. Estrus was detected with a teaser bull and artificial insemination

was carried at first detection of estrus and again 12 h later. Buffaloes not observed in estrus up to 24 h after second GnRH injection were fixed time inseminated at 24 h and 36 h after the second GnRH injection. One out of three (33 %), two out of three (66.66 %) exhibited estrus within 24 h in Category-I and II respectively, after treatment. The pregnancy rate was 33.33 percent and 66.66 percent in Category-I and II whereas 0 percent in Category-III, respectively.

Vijayrajan *et al.* (2007) selected forty repeat breeding buffaloes on the day of estrus and assigned equally to four groups namely Group-I (control), Group-II (GnRH+ PGF₂α), Group-III (Ovsynch 33) and Group-IV (Ovsynch 48). Buffaloes from Group-I were inseminated whereas buffaloes from remaining groups were injected with 10 ug of GnRH analogue (Buserlin acetate) and 25 mg of PGF₂α (Dinoprost tromethamine) intramuscularly on day 5 and 12 of the estrus cycle, respectively. Buffaloes from Group-II were inseminated only at detected estrus. Buffaloes from Group-III and IV received a second GnRH injection at 33 and 48 h after PGF₂α treatment, respectively and A. I. was carried out at 16 to 18 h after the second GnRH injection in these two groups. The interval of estrus after PGF₂α was 55.3±2.11, 55.3±2.37 and 51.8±2.49h in Groups-II, III, and IV, respectively while percentage of induced estrus was 80, 100 and 100 percent in Group-II, III and IV, respectively. The first service conception rate was 30, 50, 30 and 40 percent, respectively in Group-I, II, III and IV, respectively

Roy and Prakash (2008) treated eleven buffaloes with Ovsynch protocol i.e. administering 10 ug of GnRH analogue (Buserlin acetate) at any stage of estrus cycle (d 0) followed by 25 mg PGF₂α (Dinoprost tromethamine) on (d 7) and second GnRH treatment 48 h after PGF₂α administration and fixed time A.I. at 12 h and 24 h post second GnRH administration. Following Ovsynch treatment, 82 percent (9/11) buffaloes exhibited estrus.

Warrich *et al.* (2008) studied the timing of ovulation and pregnancy rate in Niliravi buffaloes in PGF₂α induced luteolysis and Ovsynch protocol. The mean time of ovulation after the onset of standing estrus was

greater (30.6 ± 1.5 h) in $\text{PGF}_2\alpha$ induced luteolysis compared to Ovsynch protocol (15.0 ± 8 h). Pregnancy rate of buffaloes bred at detected estrus and in Ovsynch protocol was 62.5 percent and 36.35 percent in breeding season, whereas 55.5 percent and 30.4 percent in low breeding season, respectively.

Ghuman *et al.* (2009) treated eleven true anestrus buffalo heifers in summer season with Ovsynch protocol. Ovsynch protocol involved administration of GnRH analogue at days 0 and 9, and $\text{PGF}_2\alpha$ analogue on day 7. Buffalo heifers were inseminated after 24 h interval till ovulation and simultaneously estrus symptoms were recorded. All the eleven buffalo heifers (100 percent) exhibited estrus and estrus symptoms persisted for 24 h (n=9) or 48 h (n=2). The first service conception rate was 18 percent ($p < 0.05$) in Ovsynch protocol.

Hammam *et al.* (2009) divided twenty nine Egyptian buffalo heifers into three treatments groups Heifers from Group-I were injected with GnRH (2.5 ml Receptal) followed 7 days later by $\text{PGF}_2\alpha$ (5 ml Lutalyse) and after 48 h injection with eCG (1000 I.U.) intramuscularly. Eleven buffalo heifers from Group-II were administered with GnRH (2.5 ml Receptal), $\text{PGF}_2\alpha$ (5 ml Lutalyse) and GnRH (2.5 ml Receptal) at 0, 7 and 9 days, respectively. Eight heifers from Group-III (control) were not treated for synchronization of estrus. The estrus was detected twice daily at 12 h interval by experience herdsman for at least 1 h for estrus signs. The proportion of heifers exhibiting estrus and pregnancy rate were 70; 42.8 percent for Group-I and 63.6; 57.1 percent for Group-III whereas 0 percent for control non-treated group, respectively.

Qin *et al.* (2009) selected forty seven swamps and crossed water buffaloes at Guangxi Research Institute, National Water Buffaloes Trial Farm. All the selected buffaloes were ranged from 3 to 8 year old with normal estrus cycle and free from any reproductive disease. All the selected buffaloes were synchronized by receiving 10 ug of GnRH analogue (Fertgyl) to the buffaloes irrespective of the stage of estrus cycle (d 0), followed by 20 ug of $\text{PGF}_2\alpha$ (Lutalyse) (d 7), and second GnRH treatment 10 ug 48 h after $\text{PGF}_2\alpha$ (d 9). Estrus was detected twice daily using two vasectomised bulls. The

number of buffaloes exhibited estrus were 91.7 (43/47) percent.

Roy and Prakash (2009) treated twenty two repeat breeding Murrah buffalo heifers with Ovsynch and Ovsynch plus norprolac treatment schedule and TAI was carried out 12 and 24 h post second GnRH injection. Along with Ovsynch protocol, norprolac (10 mg/animal i.m.) was administered daily for ten days in Ovsynch plus norprolac protocol. Out of eleven, five buffalo heifers (45.45 per cent) found pregnant in Ovsynch protocol whereas six out of eleven (54.54 per cent) buffalo heifers found pregnant in Ovsynch plus norprolac protocol.

Chaikhunab *et al.* (2010) selected ninety eight swamp buffaloes and divided into two groups and fifty five buffaloes (heifers n=20 and cows n=35) were synchronized with GnRH on day 0 followed by PGF₂ α on day 7, and second treatment with GnRH on day 9. All the treated buffaloes were inseminated at 12 h and 24 h after second injection of GnRH. Group-II of 43 buffaloes (heifers n=19 and cows n=24) were not treated and inseminated at natural estrus. In the Ovsynch treatment group, the overall pregnancy rate was 32.7 percent whereas 34.9 percent in control group.

Dharani *et al.* (2010) synchronized Murrah buffaloes maintained at the ideal and identical stall fed condition by the Centralized Embryo Biotechnology Unit, Chennai. In the experiment-I, six buffaloes received 8 ug of GnRH (Buserlin acetate) on day 6 of the estrus cycle followed by 25 mg of PGF₂ α (Dinoprost tromethamine) on day 13 and second dose of 8 ug of GnRH at 48 h after the injection of PGF₂ α . The Ovsynch protocol was initiated on the growth phase [OVGP] on the dominant follicle of the first follicular wave. In experiment-II instead of growth phase, the Ovsynch protocol was initiated on regression phase [OVRP] of the dominant follicle of first follicular wave i.e. day 10 of the estrus cycle. The number of buffaloes ovulated after second GnRH injection were 6/6 (100 percent) and 2/6 (33.33 percent) while interval between injection and ovulation was 24-48 h in OVGP and OVRP protocol, respectively.

Girhepunge *et al.* (2010) selected thirty buffaloes from field condition and divided in three Groups (n=10). Buffaloes from Group-II were synchronized with two injection of injection PGF₂α (Cyclix) 2 ml intramuscularly 11 days apart while buffaloes from Group-III with Ovsynch protocol whereas, buffaloes from Group-I kept as control without any hormonal treatment. The average duration required for onset of estrus was 177±24.50, 60.80±2.86 and 70.62±14.82 h in Group-I, II and III, respectively. The conception rate was 10, 30 and 40 percent in Group-I, II and III, respectively.

Karen and Darwish (2010) studied the efficacy of Ovsynch protocol in cyclic and noncyclical Egyptian buffaloes in summer. A total twenty one Egyptian pluriparous buffalo cows (11 cyclic and 10 acyclic) were selected and administered 100 ug GnRH i/m at day 0, 500 ug PGF₂α i/m at day 7, and 100 ug GnRH i/m at day 9. The buffalo cows were inseminated at 16 to 20 h after second GnRH administration. Nine out of eleven, 81 percent (9/11) of cyclic and 60 percent (6/10) acyclic buffalo cows ovulated after administration of second GnRH injection, respectively. The conception rate was 18 percent (2/12) and 0 percent (0/10) in cyclic and acyclic buffalo cows, respectively.

Kumar *et al.* (2010) synchronized thirty four post partum buffaloes maintained under village condition in loose housing system. All the buffaloes were treated with Ovsynch treatment schedule, Receptal 10µg (day 0), Cloprostenol 20 ug 7 days later and Receptal 10 µg (day 9) intramuscularly. The buffaloes were detected in estrus daily two times (at morning and evening) for onset of estrus. The buffaloes detected in estrus were bred by natural services using fertile bull while pregnancy was diagnosed per rectum 50 days post service. Out of 34 treated buffaloes, 28 (85.35 per cent) exhibited estrus. The time required for onset of estrus following treatment was 3.08 ± 0.23 day and first service conception rate was 53.57 percent.

Oropeza *et al.* (2010) selected eighty multiparous lactating Murrah buffaloes with at least 45 day postpartum and milk production > than 5 kg per day. The selected buffaloes were divided in Ovsynch protocol (n =40)

and presynch-Ovsynch (n=40) synchronization protocol. Buffaloes in the Ovsynch protocol were treated with 10 ug of GnRH intramuscularly (Buserlin acetate) and 0 followed by 25 mg of PGF₂α (Dinoprost tromethamine) on d 7 and second injection of 10 ug GnRH on d 9. Buffaloes in the presynch-Ovsynch protocol received two PGF₂α injection 14 days apart with second injection administered 14 days prior to the start of the Ovsynch protocol. All the buffaloes were inseminated 16-20 h after the second GnRH treatment. The ovulation rate was 90 percent (30/40) and 84 percent (34/40) whereas conception rate was 39 percent and 53 percent in Ovsynch and presynch-Ovsynch group, respectively.

Sharma *et al.* (2010) synchronized nine cyclic lactating Murrah buffaloes with Ovsynch synchronization treatment during summer season (day 0-GnRH; day 7- PGF₂α –; day 9-GnRH). All the buffaloes showed ovulation after second GnRH treatment. The mean diameter of ovulatory follicle at induced estrus was 13.45 ± 0.82 mm while it was 15.08 ± 0.64 mm at subsequent estrus.

Navarange *et al.* (2012) synchronized twelve normal cyclic buffaloes by Ovsynch protocol consisting 2.5 ml GnRH (Receptal) on day 0, 2 ml PGF₂α (Cyclic) on day 7 and 2.5 ml GnRH (Receptal) on day 9. The buffaloes were inseminated after 16 to 20 h after second dose of injection GnRH. All the twelve (100 per cent) buffaloes exhibited estrus and the pregnancy rate was 41.66 per cent.

Thorat *et al.* (2012) studied fertility potential in postpartum Marathwadi anestrus buffaloes using ovsynch and select synch protocols. Total twenty four Marathwadi buffaloes with absence of heat since 60 – 90 days postpartum period were selected and divided into three groups. In group I, (Ovsynch protocol), the buffaloes (n=8) were treated with intramuscular inj. of GnRH@ 10 ug on day 0 followed by inj.PGF₂α @ 500 ug on the day 7, second inj. Of GnRH @ 10 ug was administered on the day 9. In group II (Selectsynch protocol) the buffaloes (n=8) were treated with i/m inj. of GnRH@ 10 ug on day 0 followed by inj.PGF₂α @ 500 ug on the day 7. No treatment was given to

the buffaloes in control group III. Animals confirmed in estrus were bred by natural service. In group I, out of eight treated buffaloes, seven buffaloes exhibited estrus (87.50%) and the estrus in duction interval and duration of estrus were found 41.25 ± 2.26 and 11.25 ± 0.73 hours, respectively while conception rate was 85.71%. In group II, out of eight treated buffaloes, six buffaloes exhibited estrus (75.00 per cent) and estrus in duction interval and duration of estrus were found 29.50 ± 1.65 and 12.25 ± 0.73 hours while conception rate was 83.33%, respectively. In control group, out of eight treated buffaloes, only one buffalo exhibited estrus (12.50.per cent) and duration of estrus was 10.50 hours. The overall conception rate was 12.50 per cent in control group.

Savalia *et al.* (2014) studied influence of controlled breeding techniques on fertility in true anestrus and repeat breeding buffaloes. In this experiment, total fifty multiparous 90-180 days postpartum buffaloes having average body condition score of which twenty true anestrus, twenty repeat breeders and ten normal cyclic (control) were selected. Twenty anestrus buffaloes were treated initially once intramuscularly with Sodium acid phosphate 2gm and multivitamin AD₃E injection 10 ml. Ten anestrus buffaloes each were treated either with standard controlled internal drug releasing (CIDR) intravaginal device and Ovsynch (GPG) protocols with fixed time artificial insemination (FTAI). Ten repeat breeding buffaloes with mature mid-cycle palpable corpus luteum (CL) were treated with i/m injection of 25 mg prostaglandin F₂ α (PGF₂ α) with FTAI twice at 72 and 96 h later, whereas other ten repeat breeding buffaloes in standing estrus were inseminated with simultaneous i/m injection of gonadotropin releasing hormone (GnRH) 20 μ g. Ten buffaloes exhibiting first estrus within 90 days postpartum and inseminated without any treatment served as normal cyclic control. CIDR and Ovsynch protocols resulted in 100 and 80 per cent induction of estrus with conception rates of 40 and 30 per cent at induced estrus, respectively in anestrus buffaloes. Mid-cycle PGF₂ α treatment resulted in 90 per cent estrus induction and 40 per cent conception rate at induced estrus, while buserelin acetate-GnRH 20 μ g injection at AI resulted in 30 per cent conception rate in

repeat breeders. In normal cyclic control group, the first service conception rate was 30 percent.

Nakrani *et al.* (2014) studied efficacy of controlled breeding techniques in anestrus buffaloes. In this experiment, total fifty-five buffaloes more than ninety days postpartum anestrus having smooth inactive ovaries were selected. All selected buffaloes were initially dewormed using Ivermectin 100 mg s/c and owner of animals were supplied with multi mineral boluses for oral supplementation to their animals @ one bolus daily for seven days. All anestrus buffaloes were randomly subjected and treated with three standard hormonal protocols (CIDR, Ovsynch and Crestar, n=15 each), keeping a group of untreated anestrus control (n=10) and a group of normal cyclic control (n=10). All the fifteen (100percent) buffaloes in each group under CIDR, Ovsynch and Crestar protocols exhibited induced estrus within mean intervals of 65.00 ± 1.55 , 69.46 ± 1.04 and 46.00 ± 1.37 hrs, respectively, from PGF 2α injection. The conception rates obtained at induced estrus with CIDR, Ovsynch and Crestar protocols were 46.67, 53.33 and 33.33 per cent, respectively. In untreated anestrus control (n=10), only 2 buffaloes exhibited spontaneous estrus within 90 days of follow up and one (50 per cent) conceived at first A.I. In normal cyclic control group, the conception rates at first, second and third cycle and overall three cycles were 40.00, 33.33, 25 and 70 per cent, respectively.

Gupta *et al.* (2015) studied fertility response in postpartum anestrus buffaloes using modified Ovsynch based timed insemination protocols. In this experiment, total 50 post-partum anestrus dairy buffaloes were selected and randomly divided into five groups, each comprising ten animals (n=10). Animals of Group I (Ovsynch protocol) were administered i/m buserelin acetate 10 μ g at day 0 followed by cloprostenol 500 μ g on day 7 and second dose of GnRH (10 μ g) on day 9. In Group II (Modified Ovsynch protocol I) administered i/m buserelin acetate 20 μ g at day 0 followed by cloprostenol 500 μ g on day 7 and second dose of GnRH (10 μ g) on day 9. In Group III (Modified Ovsynch protocol II) administered i/m buserelin acetate 10 μ g at day 0 followed by Insulin @ 0.25 I.U/kg body weight s/c on day 0,1

and 2 and Cloprostenol (500 ug) i/m on day 7 and second dose of GnRH (10ug) on day 9. In group IV (Modified Ovsynch protocol III) administered i/m buserelin acetate 20 µg at day 0 followed by Insulin @ 0.25 I.U/kg body weight s/c on day 0,1 and 2 and cloprostenol (500 ug) i/m on day 7 and second dose of GnRH (10ug) on day 9. In group V administration of insulin on day 0,1 and 2, followed by cloprostenol (500 ug) i/m on day 7 and GnRH (10 ug) on day 9. Animal of all groups were inseminated at fixed time using frozen semen at 60 h and 72 h after PGF₂α administration and confirmed for pregnancy at day 60 post-insemination. Fertility response in terms of estrus synchronization and conception rate were recorded 60 and 20 per cent in group I, 70 and 40 per cent in group II and 80 and 40 per cent each in group III, IV and V, respectively.

Buhecha *et al.* (2016) studied efficacy of various ovulation synchronization protocols in anestrus buffaloes. A total forty six postpartum true anestrus buffaloes more than 90 days and ten normal cyclic control buffaloes that exhibited spontaneous estrus within 90 days postpartum were selected. All identified animals were dewormed by using Inj. Ivermectin 100 mg s/c and were treated initially with i/m inj. of inorganic phosphorus and multivitamin AD₃E 10 ml and oral multi-minerals one bolus daily for 7 days. All anestrus buffaloes were randomly subjected to standard estrus synchronization protocols (TriU-B, Ovsynch and Heatsynch, n=12 each), keeping a group of untreated anestrus control (n=10) and a group of normal cyclic control (n=10). In anestrus buffaloes under Tri U-B, Ovsynch and Heat synch protocol, estrus induction rate was 83.33, 83.33 and 91.66 per cent within mean interval of 69.30±0.80, 70.60±1.30 and 69.20±1.49 hr from PG injection while conception rate obtained at induced estrus was 25.00, 33.33 and 25.00 per cent and overall conception rates of three cycles were 50.00, 58.33 and 50.00 per cent, respectively. In untreated anestrus control group, out of ten buffaloes only two (20per cent) buffaloes expressed spontaneous estrus after forty one and sixty three days of initiation of experiment and conception rate was 20 per cent. In normal cyclic control group, conception rate at first and overall three cycles was 30.00 and 50.00 per cent, respectively.

Khasatiya *et al.* (2017) studied the estrus induction and conception rate in 18 postpartum anestrous Surti buffaloes divided equally into three groups and treated with Ovsynch protocol alone and in combination with PRID, keeping one group as control. The use of Ovsynch alone and Ovsynch + PRID protocols with fixed-time artificial insemination resulted in 100% estrus induction with 66.66% and 50.00% conception rate at induced estrus, respectively, and the overall conception rate was 83.33% in both the groups. In untreated anestrous control (n=6), only four (66.66%) buffaloes exhibited spontaneous estrus within 120 days of follow-up and conceived giving the first service and overall conception rate as 75.00% and 66.66%, respectively. The mean number of services per conception did not differ significantly among the treatment and control groups. The service periods of buffaloes under both the treatment groups were significantly.

Rabidas and Gofur (2017) conducted the study to evaluate estrus responses and conception rate in response to the fixed time artificial insemination of indigenous buffaloes (n=97) with Ovsynch protocol. Buffaloes (morning and evening groups) were synchronized in three different seasons of Bangladesh. Diagnosis of conception was performed by real-time transrectal ultrasonography using an ultrasound scanner on Day 40 post-AI. Ovsynch treatment induced the estrus signs in all buffaloes. The conception rate was 56.70% (55/97) and pregnancy rate was 31.96% (31/97) in indigenous river type buffaloes of Bangladesh. Though the difference of conception and pregnancy rate was insignificant ($p>0.05$) among seasons, the conception rate was varied from 44% to 64.87% and the pregnancy rate varied from 28% to 35.14% in different seasons of Bangladesh. The conception rate was also varied depending on the time of administration of hormones as well as the time of insemination. The conception rate was the highest (64.87%) in late autumn (Hemonto) season when the temperature was low (17-22°C) and the higher in morning group (61.54%). Hence they concluded that the Ovsynch protocol effectively synchronized estrus in indigenous river type buffaloes with a reasonable conception rates after fixed time artificial insemination.

Singh *et al.* (2017) the objective of the study was to compare conception rates in 42 postpartum anoestrous buffaloes following Ovsynch and Doublesynch treatments under field conditions. The buffaloes were randomly divided into 2 groups based upon the type of treatment viz. Ovsynch (n=20) and Doublesynch (n=22) groups. All animals were inseminated at 14-16 h later following last injection of respective treatment and tested for pregnancy at 60 days later. In Ovsynch group, buffaloes with no corpus luteum (CL) at the time of initiation of treatment did not conceive; however, in Doublesynch group 6 (50.0 %) buffaloes got conceived. The conception rates in buffaloes having CL at the commencement of treatment were 40.0 and 70.0 % in Ovsynch and Doublesynch groups, respectively. The overall conception rate was higher ($P<0.05$) in Doublesynch (59.1 %) compared to Ovsynch (20.0 %) group. In conclusion, Doublesynch treatment may affectively be applied to treat anoestrous in postpartum buffaloes irrespective of their ovarian status.

Arun (2018) conducted the study on pluriparous Jaffarabadi buffaloes (24) which were divided into two groups. Control group was inseminated twice at 24 h interval during natural estrus with good quality frozen thawed semen. Whereas treatment group buffaloes were treated with Ovsynch protocol. It is observed that treatment group were achieved significant higher pregnancy rate when compare to control group.

Prasad *et al.* (2019) conducted the study to evaluate the efficacy of Ovsynch and double PG protocols in postpartum anoestrous graded Murrah buffaloes during the period October 2017 to December 2017. The selected animals were randomly divided into three groups, Group I: Ovsynch protocol (n=10) having smooth ovaries, Group II Double PG protocol (n=10) the animals that are cyclical and subestrus, Group III control (n=10) normal cyclic without any hormonal intervention. All the animals were initially dewormed with Oxyclozoxide and supplemented with mineral mixture. Before initiation of treatment, blood samples were collected for estimation of serum calcium and phosphorus. They observed the in Group III animals estrous response was recorded as 60 %. Conception rate was higher in Group I (60%) than in Group

II (50%) and Group III (33%). Furthermore, no significant difference was recorded in the levels of serum calcium and phosphorus in between the groups.

2.2. Double PGF₂ α treatment in Ovsynch protocol

Carvalho *et al.* (2015) studied that a double PGF₂ α injection during the Ovsynch protocol is able to boost luteolysis. In their study, the authors divided cows into four groups. Their first group's regimen was OvSynch without any modification, the second group received GnRH presynchronisation, the third group was medicated with Ovsynch with double PGF₂ α injection, and the 4th group was assigned a combination of the two previous protocols. The pregnancy rates per artificial insemination (P/AI) by group were 32%, 33%, 37%, and 40%, respectively. Cows treated with double PGF₂ α injections and with lower P4 concentration on the day of TAI had better reproductive outcomes than cows from the 3rd and 4th groups. The recommended time for the second PGF₂ α injection is 24 h after the first injection without any changes to the times of GnRH injection or TAI.

Wiltbank *et al.* (2015) evaluated the effect of a second treatment with prostaglandin F₂ α (PGF) during Ovsynch on regression of the corpus luteum (CL) and on fertility to the timed artificial insemination. They used 2,148 lactating dairy cows on 11 dairy farms in 4 different regions of the United States. Cows were synchronized with Ovsynch and received timed artificial insemination at 60 \pm 3d in milk. Cows that received second dose of PGF had a tendency for increased pregnancies per artificial insemination (P/AI) compared with cows with single dose of PGF (36.1 vs. 33.3%) after combining data from the 2 experiments indicated a 9.45% relative increase in P/AI for cows receiving second dose compared with single dose PGF (37.6 vs. 34.4%) with the increase in P/AI observed in multiparous but not in primiparous cows. Thus, a second PGF treatment in Ovsynch type protocols can increase pregnancy success by about 10%, primarily due to enhanced fertility in multiparous cows.

Borchardt *et al.* (2018) reviewed the literature with the objective of evaluating the effects of adding a second PGF_{2α} treatment during the Ovsynch protocol on luteal regression and reproductive performance in lactating dairy cows. He stated that adding a second PGF_{2α} treatment on day 8 during the Ovsynch protocol increased the relative risk (RR) of complete luteal regression at the end of the Ovsynch protocol (relative risk=1.14;95% confidence interval=1.10 to 1.17) using a fixed effects model and the relative risk for pregnancy (relative risk = 1.14; 95% confidence interval = 1.06 to 1.22) 32 days after TAI using a fixed effects model. In summary, they observed that there was a clear benefit of an additional PGF_{2α} treatment during the Ovsynch protocol on luteal regression (+11.6 percentage units) and on P/AI (+4.6 percentage units).

Morini *et al.* (2019) investigated the fertility of dairy cows during summer season after treatment with a single or double PGF_{2α} in progesterone (P4)-based Ovsynch protocol for fixed-time artificial insemination (FTAI). Overall pregnancy rate at FTAI was lower during summer compared with winter season (30% vs 41.7%; P<0.05). During summer the percentage of pregnant cows that became pregnant at FTAI after treatment with double or single PGF_{2α} was not different while cumulative-PR at day 60 after calving was significantly (P<0.05) improved in the group treated with double compared to single PGF_{2α} treatment (42.9% and 69% for PGF₂ and 2PGF_{2α} cows, respectively). During the winter season no differences were observed in PR and cumulative-PR after single or double PGF_{2α} administration.

Nowickiv *et al.* (2019) evaluated the effect of a second prostaglandin F_{2α} (PGF_{2α}) treatment during Ovsynch on luteal regression and fertility in dairy cows, compared with standard Ovsynch. The study was conducted on 111 Holstein Friesian multiparous cows on commercial dairy farm. The cows in the experimental group (n=48) received two treatments of PGF_{2α} 24 hours apart during Ovsynch. The cows in the control group (n=63) were synchronized with standard Ovsynch. To assess the progesterone (P4) concentration blood samples were collected at the day of PGF_{2α} treatment and

at the second GnRH treatment. Pregnancy was evaluated by ultrasound examination 37-40 days after timed artificial insemination (TAI) by ultrasound. Cows diagnosed pregnant were re-examined between days 70-80 after TAI. The percentage of cows with complete corpus luteum (CL) regression ($P < 0.05$) in the pregnancies per artificial insemination (P/AI) between the experimental and control group (P/AI). However, the pregnancy loss rate was lower in cows receiving two PGF 2α treatments than in the control animals.

Rheinberger *et al.* (2020) compared fixed time artificial insemination (FTAI) conception rates and serum progesterone concentrations at the time of FTAI for cows treated with the original Ovsynch program with the same measures for cows receiving a modification of the Ovsynch program that involved a second injection of PG on Day 8. The control group ($n = 851$) received the original Ovsynch program: Day 0 GnRH, Day 7 prostaglandin F 2α , Day 9 GnRH and fixed-time artificial insemination at Day 10, approximately 13 to 16 hours after the second GnRH injection. The treatment group ($n = 852$) received a modified Ovsynch program with an additional prostaglandin injection on Day 8. Subsets of cows from each group were sampled for blood progesterone at the time of artificial insemination. Cows receiving the modified Ovsynch program had unadjusted FTAI. Conception rates that were 7% (95% CI 2%-12%) greater than cows receiving the original Ovsynch program. After adjusting for age, days in milk at mating start date and herd, the odds of conception to FTAI was significantly greater for modified Ovsynch cows (OR = 1.36, 95%CI 1.12-1.66) compared with original Ovsynch cows.

Borchardt *et al.* (2021) evaluated the effects of adding a second PGF 2α treatment during the Ovsynch protocol on P/AI in lactating dairy cows. There was a clear benefit of an additional PGF 2α treatment during the Ovsynch protocol on P/AI. Despite additional costs for hormones and labor, an additional treatment with PGF 2α on d 8 was more profitable in 95% of all scenarios because of the associated increase in fertility. It was observed that addition of second PGF 2α treatment was more profitable in multiparous cows compared with primiparous cows.

Minela *et al.* (2021) studied the effect of a double dose of Cloprostenol sodium (1.0 mg) on fertility in cows. Cows were allocated to receive 1 of 3 treatments negative controls 0.5 mg of Cloprostenol sodium (single; n = 337); positive controls: two 0.5 mg doses of Cloprostenol sodium 24 h apart (two/24; n = 313); and treated: 1.0 mg of Cloprostenol sodium (double; n = 298) at the final PGF_{2α} of Double-Ovsynch. Cows received artificial insemination (AI) 16 h after final GnRH of Double- Ovsynch. Pregnancy diagnosis was determined at 24, 34, 62, and 184 days post- AI. No evidence was observed of an effect of treatment on pregnancy rate as well as pregnancy losses per AI at 24, 34, or 62 days post-AI. However, third-plus parity cows treated with the single treatment had greater pregnancy loss compared with two/24 and double between days 24 and 34 post-AI.

Tippenhauer *et al.* (2021) evaluated the effect of two prostaglandin F_{2a} (PGF) treatments 24 h apart (500 mg of Cloprostenol) and treatment with a double PGF dose on d 7 (1000 mg of Cloprostenol) during a 7-d Ovsynch protocol on progesterone (P₄) concentration and pregnancy per artificial insemination (P/AI) in lactating Holstein cows. The experiment was conducted on 8 commercial dairy farms in which cows were treated with Ovsynch (Control), Ovsynch with double PG dose on day 7 (GDPG) and Ovsynch with second PG treatment 24 hour later (GPPG). Timed artificial insemination was carried out approximately 16 hour after second GnRH injection. Pregnancy per artificial insemination (P/AI) was 31.8% (n=888) in Ovsynch protocol, 33.4% (n=814) in GDPG protocol while 40.3% (n=871) in GPPG protocol, respectively. It was concluded that addition of second PGF treatment on d 8 during 7 day Ovsynch protocol increased P/AI compared to Ovsynch protocol and to double PGF dose on day 7.

2.3. Estrual cervical mucus properties

2.3.1. Hydrogen ion concentration (pH)

Pattabiraman *et al.* (1967) observed the pH of cervical mucus from total 58 healthy and regular breeder cows of which 25 in early, 25 in mid and 8 in late estrum and found that 8, 44, 48 per cent and 8, 76 and 16 per cent animal showed pH less than 7, 7 to 9, and more than 9 in early and mid estrus,

respectively. In late stage of estrus out of eight, 0, 87 and 18 per cent animals showed pH less than 7, 7 to 9, and more than 9, respectively. The pH of cervical mucus from healthy and regular breeder cows fell within range of 7.0–9.0 with the mean of 8.03 ± 1.22 .

Shehata *et al.* (1978) observed pH of cervical mucus in 54 cows and 46 buffaloes during estrus and pooled samples of cervical mucus during luteal phase. The mean pH was 7.51 ± 0.1 and 7.46 ± 0.071 during estrus and 6.65 and 6.5 during luteal phase in cows and buffaloes, respectively.

Wani *et al.* (1979) studied pH of cervical mucus of total 40 adult healthy cross bred cows. Normal conceived cows and those cows which did not conceive within three services were taken as repeaters were divided into two groups. The mean pH of cervical mucus was 6.97 ± 0.24 and 7.61 ± 0.16 in normal and repeater cows, respectively.

Gupta *et al.* (1981) studied pH of vaginal mucus of 13 normal and 6 repeat breeder pluriparous Rathi cows and found that the mean pH was 7.8 ± 3.60 and 8.5 ± 2.45 in normal and repeat breeder cows, respectively.

Rao and Rao (1982) studied the pH of oestrial mucus in 41 samples obtained from crossbred heifers and observed that most of samples were found to be alkaline reflecting a change in hydrogen ion concentration (pH) influence the fertility. The pH of cervical mucus was found to range from 7 to 8 with mean of 7.93 and appeared to be closely associated with fern pattern.

Pandey *et al.* (1983) studied pH of cervical mucus of 22 repeat breeding and 32 normal-control cows and reported that pH of cervical mucus obtained from repeater cows was 7.20 as compared to 7.38 in control cows.

Singh and Kharche (1984) studied pH of cervico-vaginal mucus from 72 estrus periods of 56 normally cycling crossbred cows at midheat stage and divided in three estrus intensity groups i.e. intense, intermediate and weak. The average pH was 7.25 ± 0.07 , 7.18 ± 0.04 and 7.57 ± 0.16 in intense, intermediate and weak estrus group, respectively.

Umashanker *et al.* (1984) studied pH of cervical mucus in 20 cross bred estrus cows and divided into two groups namely normal cyclic and repeat breeding cows. The mean pH of normal cyclic cows was 6.90 ± 0.49 and 8.2 ± 0.26 in repeat breeding cows.

Adhalikar (1986) studied the pH of cervico-vaginal mucus at mid-estrus from ovulated and anovulated cross bred cows and from ovulated animals with fertile heat, infertile heat and heat in repeat breeders. The hydrogen ion concentration (pH) was higher in ovulated cows than in anovulated cows and in ovulated groups the values were lower in fertile heat than in infertile and heat in repeat breeders, respectively.

Salphale *et al.* (1993) studied the pH of estrual cervical mucus of 9 normal as well as repeat breeder crossbred cows. All cows were examined at least one regular estrus and later cows were synchronized with single injection of Dinofertin 25 mg i/m. Samples were collected at mid stage of regular and induced estrus. No significant difference in the pH of cervical with fertile (8.00 ± 0.078) and infertile estrus (7.98 ± 0.084) was observed.

Modi *et al.* (2011) studied pH of cervico-vaginal mucus of 10 normal and 20 repeat breeder Kankrej cows. The average pH of cervical mucus of normal cows was 8.39 ± 0.17 with a range of 7.3 to 9.1 whereas values varied from 4.7 to 7.9 with average of 6.19 ± 0.18 in repeat breeder crossbred cows. The normal breeder showed higher alkaline pH as compared to repeat breeder crossbred cows and alkaline pH of cervical mucus was more favourable for sperm progressive motility.

Verma *et al.* (2014) studied pH of cervical mucus in 94 Murrah buffaloes and categorized into three groups ranges between 7 to 7.5, 7.5 to 8 and more than 8. Cervical mucus of 68.09 per cent estruses were within pH range of 7.5 to 8.0 while 19.15 per cent and 12.76 per cent cervical mucus were in pH range of >8 and 7.0-7.5, respectively.

Wankar, M. S. (2016) studied mean pH of cervical mucus in 12 buffalo divided into two groups (Group-I (Clomiphene citrate), Group-II (Ovsynch protocol) was found to be 7.36 ± 0.12 in group-I (Clomiphene

citrate) and 7.53 ± 0.15 in group-II (Ovsynch Protocol). The result was statistically non significant at ($p < 0.01$) level.

2.3.2. Fern pattern

Galhotra *et al.* (1971) studied the arborization pattern of cervical mucus in total 46 buffalo heifers and found 30 (65.21 per cent), 11 (23.91 per cent) and 5 (10.86 per cent) were with typical, atypical and no pattern category of fern like crystal, respectively. The number of buffalo heifers ovulated was 38 (82.60 per cent) and conceived was 34 (73.91 per cent).

Shehata *et al.* (1978) examined fern pattern of 46 sample of cervical mucus secretion of buffaloes during estrus and pooled samples of cervical mucus during luteal phase. During estrus typical and complete fern patterns observed in 38 (82.60 per cent) samples and incomplete typical in 8 (17.39 per cent) samples. Pooled samples of cervical secretion of buffaloes during luteal phase gave no patterns.

Bishnoi *et al.* (1982) studied fern pattern of the cervical mucus of 26 normal and 26 repeat breeder cows in estrus and reported typical and atypical types of fern pattern. The per cent of fern pattern in normal cows was 96 for typical and 4 for atypical while in repeat breeder cows the per cent was 38 and 62 for typical and a typical, respectively. The overall pregnancy rate was 69.2 and 38.4 per cent in normal and repeat breeder cows.

Rao and Rao (1982) studied fern pattern of estrual mucus in 41 samples obtained from crossbred heifers and reported typical fern pattern in 85.37 per cent of cases and atypical fern pattern was most commonly observed in cloudy and alkaline samples.

Pandey *et al.* (1983) studied fern pattern of cervical mucus of 22 repeat breeding and 32 normal-control cows and reported that ferning pattern in cervical mucus obtained from repeater cows and control cows was not significantly different.

Danell *et al.* (1984) studied fern pattern in 29 Surti buffalo heifers. The fern pattern was classified into four types i.e. grade 3, grade 2, grade 1 and grade 0 for very characteristics, characteristics, less characteristics

and no fern pattern, respectively. Out of twenty-nine, 15 (51.7 per cent), 9 (31.0 per cent), 2 (6.9 per cent) and 3 (10.3 percent) buffalo heifers had a very characteristic (grade3), characteristic (grade 2), less characteristic (grade1) and no fern pattern, respectively.

Umashanker *et al.* (1984) studied fern pattern in 10 normal cyclic and 10 repeat breeding cross bred cows. In normal cyclic cows, typical tertiary branching was observed while in repeat breeding group, fern pattern was scattered and small size and branching was thin and fine.

Adhalikar (1986) studied the fern pattern of cervico-vaginal mucus at mid- estrus from ovulated and anovulated cross bred cows and from ovulated animals with fertile heat, infertile heat and heat in repeat breeders. The per cent of typical fern pattern in ovulatory and anovulatory heat was 75.5 and 24.5, respectively.

Sharma *et al.* (1987) studied fern pattern of cervical mucus from 43 typical repeat breeding crossbred cows. These samples tested for drug sensitivity pattern and these animals were treated with suitably selected drugs. The typical, atypical and nil arbonization pattern was noted in 26 (60.46 per cent), 52(27.91 per cent) and 5(11.63 per cent) cases, respectively before treatment where as it was 22(62.86 per cent), 10(28.57 per cent) and 3(8.57 per cent) after treatment. Conception rate was highest (90.90 per cent) in typical fern, 60 and 33.33 per cent in atypical and nil type of pattern, respectively.

Salphale *et al.* (1993) studied the fern pattern of estrual cervical mucus of 9 normal and repeat breeder crossbred cows from each group. All cows were examined at least one regular estrus and later cows were synchronized with single injection of Dinofertin 25 mg i/m. Samples were collected at mid stage of regular and induced estrus. In normal cows after synchronization, the number of cows showed typical fern pattern from seven to five (55.56 per cent) where as in repeat breeders it increased from three to five (55.56 per cent). In combined group, out of eleven fertile estrus, eight (72.37per cent) samples showed typical fern pattern while out of seven infertile estrus, three (27.28 per cent) showed typical fern pattern.

Srivastav *et al.* (2000) studied fern pattern of cervical mucus of 60 crossbred cows at A.I. and found typical 38 (63.33 per cent), atypical 16 (26.67 per cent) and nil 6 (10.00 per cent) types of pattern. Cross bred cows conceived were 21(55.26 per cent) in typical, 3 (18.75 per cent) atypical and 0 in nil type of fern pattern.

Modi *et al.* (2011) observed the fern pattern of cervico-vaginal mucus of 10 normal and 20 repeat breeder Kankrej cows. The fern pattern frequencies of typical, atypical and nil were 13 (65 per cent), 7(35 per cent) and 0(per cent) in normal whereas respective figure for repeat breeder cows were 10 (25 per cent), 22 (55 per cent) and 8(20 per cent), respectively. The atypical fern pattern was observed more in repeat breeder cows (55.00 per cent) than normal cows (35.00 percent).

Sharma *et al.* (2011) examined estrual cervical mucus samples from 61 normal cyclic buffaloes at AI. Typical, atypical and nil pattern of cervical mucus were observed in 39.34, 42.63 and 18.03 per cent samples, respectively. The number of buffaloes conceived with typical pattern was 45.83 per cent and atypical pattern was 26.92 per cent. Maximum buffaloes conceived when thick stringy mucus with typical fern pattern (44.44 per cent) followed by thick mucus with atypical fern pattern (33.33 per cent) and thin watery mucus with typical (16.16 per cent) and atypical fern pattern (5.55 per cent). The overall first service conception rate was 29.50 percent.

Verma *et al.* (2014) studied arborisation pattern of cervical mucus of 94 Murrah buffaloes and divided into three classes namely typical, atypical and nil. The cervical mucus in 51(54.25 per cent) buffaloes had shown typical arborisation pattern with primary, secondary and tertiary venation while remaining 35(37.24 per cent) and 8(8.51 per cent) buffaloes had atypical and nil arborisation patterns, respectively. The significantly ($p < 0.05$) higher conception rate (54.90 per cent) was observed in buffaloes inseminated with typical arborisation pattern.

Wankar, M.S. (2016) collected cervical mucus samples from buffalo heifers from Group-I (OvSynch synchronization protocol) and Group-II (Clomiphene citrate treatment) and classified into typical, atypical and nil type

of fern pattern. In present study, typical, atypical and nil pattern was observed in 2 (66.66 per cent), 1 (33.33 per cent) and 0(0 per cent) cervical mucus of buffalo heifers, respectively in Group-I. In Group-II, typical, atypical and nil pattern was observed in 3 (50 per cent), 2(33.33 per cent) and 1(16.66 per cent) buffalo heifers, respectively. The overall percentage of typical, atypical and nil pattern was 5(55.55 per cent), 3(33.33 per cent) and 1 (11.11 per cent) in buffalo heifers.

2.4. Pregnancy rate of sex sorted semen in buffaloes.

Doyle *et al.* (1999) conducted study on 302 Holstein-Friesian cows from a commercial dairy herd. Only cows with a corpus luteum and at least one follicle with diameter equal or greater than 10 mm at the beginning of Ovsynch protocol were included in the study. The cows with follicles with diameter between 12 and 18 mm and with clear vaginal mucus were inseminated which is a shallow uterine insemination in the uterine horn ipsilateral to the pre-ovulatory follicle. Ultrasonography examinations of the ovaries were performed during the synchronization protocol. Insemination of cows were randomized with either frozen thawed sex-sorted sperm (sex-sorted; n=148) or conventional semen (conventional; n=154). Pregnancy rate was 31.8% in sex sorted and 40.9% in conventional groups. In conclusion, insemination with sex-sorted sperm reduced fertility of lactating dairy cows subjected to a TAI program than non-sorted semen.

Seidel *et al.* (1999) inseminated 1,000 heifers consecutively with sexed sperms and 370 heifers with control sperms in 11 small field trials. In 5 of 6 field trials, there was little difference in pregnancy rates between insemination doses of 1.0 to 1.5×10^6 versus 3.0×10^6 sexed, frozen sperms.

Anderson *et al.* (2004) studied effect of insemination with doses of 2 or 15 million frozen-thawed spermatozoa and semen deposition site on pregnancy rate, without synchronization in dairy cows. A total of 284 inseminations with 2 million spermatozoa and 312 inseminations with 15 million spermatozoa were performed on 480 dairy farms. It was reported that, pregnancy rate for low dose (2×10^6) of sexed, unsexed semen was 21%, 32% and 46% for unsexed (15×10^6) AIs.

Weigel (2004) conducted field trials in Holstein and Jersey heifers by AI technicians and do-it-yourself (DIY) inseminators in commercial dairy herds. The pregnancy rate in New York by DIY inseminators in Holstein (n= 797) for sexed semen at 1.5 and 6 million sperm was 43% and 41% and for unsexed semen at 20 million sperm was 62% was recorded. In California by AI technicians in Jersey (n=637) for sexed semen at 2 million sperm was 31% and for unsexed semen at 2 and 20 million was 49% and 61% respectively. In California, Texas by DIY inseminators in Holsteins (n=513) pregnancy rate for sexed semen at 2 million sperm was 31% and for unsexed semen at 2 and 20 million was 46% and 43%, respectively.

Bodmer *et al.* (2005) performed fertility test after low dose insemination with sexed and nonsexed sperms in dairy cattle under field conditions in Switzerland. A total of 132 heifers and cows were inseminated with 2×10^6 X- bearing, frozen-thawed sperms (A) and 91 cows were inseminated with the same dose using non-sorted, frozen-thawed sperms (B). Pregnancy examination by ultrasound was performed twice, 30–40 days (PE1) and 70–90 days (PE2) after insemination. The pregnancy rates after PE1 were 33.3% (9/27) and 59.3% (16/27) in heifers and 27.6% (29/105) and 28.1% (18/64) in cows for groups A and B, respectively.

Presicce *et al.* (2005) reported first established pregnancy in Mediterranean Italian buffaloes by deposition of sexed semen near the utero tubal junction. Total twenty one buffaloes were inseminated with total four million sex sorted frozen thawed spermatozoa near the uterotubal junction ipsilateral to the ovary carrying pre-ovulatory follicle. The conception rate was 42.8 % (9/21).

Anderson *et al.* (2006) studied pregnancy rate in 306 lactating dairy cows, of which 157 were inseminated with sexed semen and 149 with conventional semen. The average pregnancy and calving rates were 21.0% and 20% for the sexed semen and 46% and 45% for the conventional semen. The proportion of female calves derived from sexed semen was 82% compared with 49% for conventional semen. The proportion of live and healthy calves in single births was 100% for sexed semen and 97% for conventional semen.

Cerchiaro *et al.* (2007) conducted experiment on 536 nulliparous Holstein heifers that were reared in 61 dairy herds of Northern Italy and were inseminated between September 2002 and April 2004. Heifers were inseminated with sperm produced by 4 proven Holstein sires (A, B, C, and D), sexed by Cogent (Chester, UK) through flow cytometry, and available under commercial conditions. Inseminations were done with either full straw, containing approximately 2×10^6 sperm, or half straw, containing 1×10^6 sperms. Heifers returning to estrus were diagnosed as not pregnant, whereas heifers that did not return to estrus were diagnosed pregnant with ultrasonography or rectal palpation. The pregnancy rate obtained using sexed cattle sperm in field conditions was slightly greater than 50%. Heifers inseminated in 2003 had a lower pregnancy rates than heifers inseminated in 2002. Several studies pointed out the negative effects caused by high humidity and high temperature on cattle fertility. The year effect, therefore, may have arisen because inseminations in spring and summer were done only in 2003. Differences in pregnancy rates among bulls for both unsexed and sexed semen were reported. These results suggest the need for careful selection of bulls based on accurate analysis of semen fertility under field conditions.

DeJarnette *et al.* (2008) collected ejaculates by artificial vagina from 3 Holstein sires and sorted to 90% purity for X-chromosome-bearing spermatozoa (range 88 to 93%) using flow cytometry. Sorted sperm were diluted to 2.1, 3.5 or 5.0×10^6 sperm per dose. Strategically allocated in 75 Holstein herds with targets for 50% use in heifers and 50% in lactating cows. Within sire A conception rates of heifers were greater for the 5×10^6 (59.5%) than for the 2.1×10^6 (46.4%) sperm dose and intermediate for the 3.5×10^6 sperm dose (52.2%). However, across sires, sperm dosage had no effect on heifer conception rates (46.7, 51.2, and 52.5% for the 2.1, 3.5, and 5.0×10^6 sperm dosages, respectively). Conception rates within sire B were numerically greater ($P = 0.06$) for the 3.5 and 5.0×10^6 sperm dosages than for the 2.1×10^6 sperm dosage, whereas sperm dosage had no effect on conception rates within sire C. In conclusion, these data indicate that an increased sperm dosage may enhance virgin heifer conception rates for some (but not all) sires, whereas neither sire nor sexed-sperm dosage affected conception rates of lactating cows.

Borchersen and Peacock (2009) compared conception rates, non-return rates and sex ratios of sexed and conventional semen from the same sires in commercial dairy herds in Denmark. Three breeds of cattle, Holstein, Jersey and Red breed were studied. The conception rate for Holstein was 12% lower for sexed semen (273/554, 49.3%) as compared to conventional (181/112, 61.9%) semen. The conception rate for Jersey was 7% lower for sexed semen (235/504, 46.6%) as compared to conventional (89/165, 53.9%) semen. The conception rate for Red breed was 5% lower of sexed (319/530, 60.2) as compared to conventional (100/153, 65.4%) semen. From all breeds, total calves born from sexed semen were 763 from which 696 are female calves and 67 are male calves. From conventional semen total 284 calves were born from which female calves were 146 and male calves was 138.

Dejarnette *et al.* (2010) compared conception rates of Holstein cows and heifers after artificial insemination (AI) with 2.1 or 3.5×10^6 sex-sorted sperms or 15×10^6 conventional sperms. Conception rate of heifers was influenced by the sire and by treatment interaction. Conception rate of the 2.1 and 3.5×10^6 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 3.5×10^6 sex-sorted sperm dosages and 15×10^6 conventional dosages were 44, 46, and 61%, respectively.

Lu *et al.* (2010) inseminated forty three buffaloes with two million spermatozoa in spontaneous estrus deeply in horn ipsilateral to the side of dominant follicle. The conception rate following AI was 69.7 per cent (30/43) for sexed semen. The conception rate was higher in heifers 77.8 % (7/9) than that of 67.6% (23/34) parous buffaloes. Out of thirty pregnancies, twenty four females and five male calves were born with one abortion.

Norman *et al.* (2010) studied the use of sexed semen for artificial insemination in US Holstein heifers (1.3 million breeding) and cows (10.8 million breeding) in Dairy Herd. Improvement herds were characterized by breeding year, parity, service number, region, herd size, and herd milk yield. Sexed semen was used for 1.4, 9.5, and 17.8% of all reported breeding for 2006,

2007, and 2008, respectively for heifers, and for 0.1, 0.2, and 0.4%, respectively for cows. The mean conception rate for heifers was 56% for conventional and 39% for sexed semen.

Campanile *et al.* (2011) reported that use of sexed semen in buffalo heifers and documented satisfactory and similar pregnancy rates when compared with conventional non sexed semen. Deposition of sexed semen into the body of the uterus, however, increased pregnancy rates significantly.

Dejarnette *et al.* (2011) compared conception rates in Holstein heifers after AI with 2.1 and 10×10^6 sperm dosages of sex-sorted and conventionally processed sperm. The conception rate of sex-sorted and conventional semen by dosage of 2.1×10^6 was 38% and 55%. and by dosage of 10×10^6 was 44% and 60% was observed.

Rhinehart *et al.* (2011) reported 38% pregnancy rates in heifers and 33% in postpartum cows using sexed semen. Heifers and postpartum cows showed no difference in fertility when inseminated with sexed semen.

Sa Filho *et al.* (2011) designed three experiments 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. In all cows ovaries were scanned by trans-rectal ultrasound at TAI and the largest follicle (LF) present was measured. In Exp. 1, a total of 853 cows had their LF classified as <9 mm or ≥ 9 mm at the time of TAI; these cows were then randomly assigned to 4 groups according to their LF diameter (<9 mm or ≥ 9 mm) and the type of sperm used (sex-sorted or non-sex-sorted). There was an interaction ($P = 0.02$) between the type of sperm and LF diameter beginning at TAI [non-sex-sorted ≥ 9 mm = 58.9%a(126/214); non-sex-sorted <9 mm = 49.5%b(106/214); sex-sorted ≥ 9 mm = 56.8%ab (134/236); and sex-sorted <9 mm = 31.2%c (59/189). In Exp.2, suckled cows ($n = 491$) were classified immediately before TAI as having displayed estrus or not

(estrus or no estrus) between P4 device removal and TAI. These cows were randomly assigned to 4 groups according to the occurrence of estrus and the type of sperm (sex-sorted or non-sex-sorted). 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)]; however, no interaction between the occurrence of estrus and type of sperm was observed ($P = 0.87$). In Exp. 3, a total of 200 suckled cows presenting LF ≥ 9 mm at TAI were randomly assigned to receive sex-sorted sperm deposited into the uterine body ($n = 100$) or into the uterine horn ipsilateral to the recorded LF ($n = 100$). No effect of deeper AI on P/AI was found ($P = 0.57$). In conclusion, the LF diameter at TAI and the occurrence of estrus can be used as selection criteria to identify cows with greater odds of pregnancy to receive sex-sorted sperm in TAI programs. In addition, performing TAI with sex-sorted sperm deeper into the uterus did not alter the pregnancy results.

Gaviraghi *et al.* (2013) reported lower pregnancy rate ($P < 0.001$) when inseminating doses of sexed semen at 2 million were used (53/170; 31.2%), with respect to conventional non-sexed (78/142; 54.9%), and sexed doses at 4, 6, and 8 million spermatozoa (102/205, 49.8%; 84/175, 48.0%; and 74/157, 47.1%, respectively). No differences were evident using conventional doses and sexed semen with sperm numbers equal or higher than 4 million per dose.

Healy *et al.* (2013) studied conception rates and other reproductive outcomes achieved with artificial insemination (AI) in nulliparous Holstein heifers using sexed and conventional semen in a commercial Australian dairy herd in central western New South Wales from January 2004 to April 2009. On-farm records of 9,870 inseminations of 4,456 heifers were analyzed using several mixed models. Semen was used from 15 sexed sires and 41 unsexed sires. 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, whereas model-based predictions were lower, at

21.3 and 32.1%. Sexed semen yielded 86% heifers, compared with 48% for conventional semen. Twinning rate was high, at 3.6% for both semen types. Abortion rates for sexed and unsexed conceptions were similar at 6.1 and 6.5%. The pregnancy rate reported was 52% to sexed semen while 58% to conventional semen. It was concluded, pregnancy rates are considerably less in dairy cows than in dairy heifers with sexed semen and is not recommended for use in lactating cows or super-ovulated females.

Mallory *et al.* (2013) compared pregnancy per AI (P/AI) with conventional (CON) or sex-sorted (SS) semen from a single sire within a fixed-time AI (FTAI) program designed for dairy heifers. Holstein heifers (n = 240) were assigned to treatment (CON or SS) according to body weight and reproductive tract score. All heifers underwent FTAI by using the “Show-Me-Synch” protocol [controlled internal drug release (CIDR) insert from d 0 to 14 followed by PGF2 α (25 mg i/m) 16 d after insert removal (d 30) with GnRH (100 μ g i/m) and FTAI at 66 h after PGF2 α]. A single professional technician performed the FTAI. Heifers were fitted with heat detection patches at PGF2 α to characterize estrous response. 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. The P/AI did not differ for CON heifers that exhibited or failed to exhibit estrus before FTAI [44/63 (70%) vs. 38/57(67%), respectively]. For SS heifers, those that exhibited estrus had greater P/AI compared with those that failed to exhibit estrus [32/70 (46%) vs. 13/50 (26%)]. Pregnancy per AI resulting from FTAI was greater for heifers that were inseminated with CON semen compared with those that received SS semen. The expression of estrus before FTAI did not affect P/AI when CON semen was used, whereas the P/AI with SS semen was greater for heifers detected in estrus.

Abdalla *et al.* (2014) evaluated the fertility of commercially available sexed semen and economically analyze three assumed strategies for its application in Holstein heifers. 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, the

embryonic loss, the calving, the abortion and the heifer calves rates were calculated. Heifers inseminated with sexed semen had significantly lower pregnancy-40, pregnancy- 90 and calving rates (34, 32.2 and 29.3%; respectively) than those inseminated with un-sexed semen (62.5, 57.8 and 51.1%; respectively). Embryonic loss and abortion rates were recorded in heifers inseminated with sexed (5.5 and 8.3 %; respectively) and unsexed semen (7.4 and 10.8%, respectively). The proportion of heifer calves born after using sexed semen (89.6%) was significantly higher than that after using unsexed semen (50%).

Karakaya *et al.* (2014) compared pregnancy per artificial insemination (P/AI) after timed AI with sex-sorted sperm (SS) or conventional semen (CS) in lactating dairy cows. The cows were inseminated with either frozen-thawed SS (n = 148) or CS (n = 154) of the same bull into the uterine horn ipsilateral to the side of probable impending ovulation. It was reported that, pregnancy per AI on day 31 tended for SS (31.8%) and CS (40.9%). Similarly, pregnancy per artificial insemination on day 62 for SS (25.7%) and CS (39.0%), respectively.

Lu *et al.* (2015) inseminated total of 4521 swamp or crossbred (F1 or F2) buffaloes with natural estrus were inseminated with X-sorted sperm from river buffaloes, resulting in 48.5% (2194 of 4521) pregnancy rate and 87.6% (1895 of 2163) sex accuracy in the derived calves. The pregnancy rate obtained with sexed semen from Murrah bulls was higher than that of Nili-Ravi, 52.5% (895 of 1706) versus 46.1% (1299 of 2815; $P < 0.01$), respectively.

Djedovic *et al.* (2016) performed 3,320 number of inseminations in which sexed and non-sexed (conventional) semen was 1,205 and 2,115, respectively and site of sperm deposition was uterine body. The conception rate was 55% for conventional and 44% for sexed semen in cows.

Kurykin *et al.* (2016) conducted experiment on 3206 heifers, housed in three free-stall barns and in four tie-stall facilities. After synchronization by two prostaglandin F2 α (PGF2 α) treatments 14 days apart, 281 heifers were inseminated conventionally and 118 intracornually with sexed

semen. At spontaneous estrus, 1129 heifers were inseminated with sexed and 529 with unsexed semen, and at estrus induced by a single PGF₂ α treatment 185 heifers were inseminated with sexed and 284 with unsexed semen. Heifers were inseminated conventionally with sexed semen 12 h after detection of estrus, and with unsexed semen according to the a.m.–p.m rule. Sexed and unsexed semen doses from five bulls were evaluated for motility, morphology, membrane integrity, and chromatin stability. Overall pregnancy rate with sexed semen was (41.7%) and with unsexed semen (51.6%) and was lower than with unsexed semen irrespective of the type and intensity of estrus, and deposition site. Insemination at spontaneous estrus resulted in a higher pregnancy rate (53.4%) than at induced (41.9%) or synchronized (44.7%) estrus. Pregnancy rates did not differ after intracornual (44.9%) or conventional insemination (48.4%). Strong estrus resulted in higher pregnancy rate (by 14.4% points) compared to weak estrus.

Shekalgorabi *et al.* (2017) compared reproductive performance of sexed versus conventional semen in 3573 heifers. The average conception rate of sex sorted and conventional semen was 48.3% and 63.8%, respectively.

Sharma *et al.* (2018) selected total 218 cows from which 70 cows inseminated with sexed semen and 148 cows with unsexed semen at mid-cervix and reported conception rate, female calves and male calves were 40%, 82.14%, 17.85%, and 49.32%, 50.68%, 49.31%, for sexed and unsexed semen, respectively.

Oikawa (2019) studied the effect of conventional and sexed semen on the conception rate in heifers. The first insemination in HF with conventional (n=41,857) and sexed semen (n=45,465) were recorded. The mean conception rate was 56.9% with conventional semen and 47.3% with sexed semen during the period of 2012 to 2016.

Patel and Jethava (2019) selected 127 heifers for breeding through sexed semen initially, of which some conceived at first AI and others showed repeat estrus after 18-21 days and were re-inseminated with sexed semen again. Thus, out of total 172 sexed semen dose utilized on 127 heifer satcal frearing project, 68 heifers conceived. The conception rate of sexed

semen found to be 39.53%. Out of these 68 pregnant heifers, 2 heifers aborted and one heifer died before calving. Out of 65 animals that calved, 54 heifers delivered live female calves, 9 heifers had male calves, and two had female stillbirth. Thus, with regards to sex of calf born with sexed semen including stillbirths, 86.15% were female calves and 13.85 % were male calves.

Sharma *et al.* (2019) reported the effect of pregnancy rate of sexed semen on different breeds for artificial insemination in females. A total of 3034 inseminations with Sexcel were performed with 2589 inseminations in dairy cows and 445 in buffaloes. The average conception rate of sexed semen in cows was 49.1% and in buffaloes 42.7% were recorded.

Dawod and Elbaz (2020) evaluated the fertility of sexed semen and conventional semen with regard to the puberty and breeding ages of Holstein dairy heifers subjected to double Ovsynch protocol with fixed time of artificial insemination. A total of 468 Holstein heifers were divided into two groups. In the first group 122 dairy heifers was inseminated via conventional semen, while in the second group 346 heifers was inseminated with sexed semen. The puberty and breeding ages of heifers were determined from the farm records. 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 a significantly lower first-service pregnancy rate (51.45%) than those inseminated with conventional semen (61.47%). Heifers achieving puberty before 350 days old had a higher pregnancy rate. Holstein heifers subjected to Ovsynch protocol with sexed semen had an acceptable first-insemination pregnancy rate.

Sawant, S. S. (2021) studied the pregnancy and calving rates after AI with sex sorted and conventional semen in Murrah buffaloes. The pregnancy rate was lower after AI with sex sorted semen than conventional semen. The average pregnancy and calving rates for sex sorted semen AIs were 40% and 100% and for conventional semen AIs were 50% and 100%, respectively. The proportion of female calves born from sex sorted semen AI was 100% and from conventional semen was 40%. The proportion of live and

healthy calves born from sex sorted and conventional semen AI was 100%. There was no difference between sex sorted and conventional semen for calving rate and health of calves.



CHAPTER - III

Materials & Methods

CHAPTER - III

MATERIALS AND METHODS

The present research work entitled “Fertility of sexed semen in Ovsynch and double PG Ovsynch protocols in buffaloes” was carried during the period of June 2021 to October 2021. This experiment was conducted at buffalo farms in Akola and at Department of Animal Reproduction, Post Graduate Institute of Veterinary and Animal Sciences, Akola.

3.1 Climatic conditions

Akola is located in subtropical region having extreme climatic condition situated at latitude of 22⁰ to 24⁰ North, longitude of 77.62⁰ East and at an altitude of 307.415 meters above the mean sea level (M.S.L.). The minimum temperature in winter and maximum temperature in summer is being 9⁰C and 47⁰C, respectively.

3.2 Source of buffaloes

Total twenty four lactating multiparous buffaloes were selected from buffalo farms in Akola.

3.3 Selection of buffaloes

The age, vaccinations of various diseases like HS, BQ and FMD and reproductive history of previous parturition were recorded of selected buffaloes. The buffaloes that have completed post-partum period of forty five days with normal reproductive genitalia and without clinical infection were selected randomly and divided into two groups (Plate 3.1).

3.4 Health and managerial practices

The selected multiparous buffaloes were dewormed using injection Ivermectin @ 1 ml per 50 kg body weight subcutaneously and initial treatment with injection vitamin AD₃E&H, 5 ml I/M and supplemented with chelated mineral mixture (1 kg) 50 gm daily orally after first gynecological examination. Feeding of greens and dry fodder along with concentrate made of

toor churi, wheat bran, crushed maize and cotton seed cakes were provided while fresh water was available ad-lib.

3.5 Experimental procedure

These randomly selected buffaloes were divided into two groups containing twelve buffaloes in each group (n=12).

Group-I (n=12)

The twelve buffalos from this group were synchronized with Ovsynch synchronization protocol (Inj. Buserlin acetate 10µg i/m on day 0, Inj. Cloprostenol sodium 500 µg on day 7 and Inj. Buserlin acetate 10µg i/m on day 9) and inseminated with sexed semen (female) after 16-20 hrs after last GnRH injection

PSMT	GnRH	PGF ₂ α	GnRH	A.I.
•	•	•	•	•
0 th day	7 th day	14 th day	16 th day	16-20 h

Group-II (n=12)

The twelve buffalos from this group were synchronized with double PGF₂α Ovsynch synchronization protocol (Inj. Buserline acetate 10µg i/m on day 0, Inj. Cloprostenol sodium 500 µg on day 7 and on day 8, Inj. Buserlin acetate 10µg i/m on day 9 and inseminated with sexed semen (female) after 16-20 hrs after last GnRH injection.

PSMT	GnRH	PGF ₂ α	PGF ₂ α	GnRH	A.I.
•	•	•	•	•	•
0 th day	7 th day	14 th day	15 th day	16 th day	16-20 h

3.6 Estrus Signs and artificial insemination

The buffaloes were observed for estrus exhibition after the last injection of PGF₂ α in both the groups and different estrus symptoms like frequent micturition, congestion of vaginal mucus membrane, vaginal discharge and tumefaction of vulva were noted by visual observations. The efficacy of estrus synchronization protocols was recorded. Artificial insemination was performed by following procedure.

3.6.1. Semen straws

Murrah buffalo bull sex sorted semen straws were purchased from Bhartiya Agro Industries Foundation (BAIF) Development Research Foundation, Uruli Kanchan, Pune (M.S.) India which were stored in Liquid Nitrogen (LN₂) till inseminations.

3.6.2. Thawing

Sex sorted semen straw of Murrah bull was taken out of the liquid nitrogen (LN₂) container using straw picking forceps, by lifting the semen containing canister below the neck level of LN₂ container. Straw was thawed by immersing immediately in thawing tray containing water of 37°C and kept for 30 seconds.

3.6.3. Artificial insemination

After thawed french mini straw were taken out, wiped with tissue paper and then factory seal of straw was cut off with scissor. The gun was loaded and AI was performed in which semen was deposited in body of uterus (Plate 3.7).

3.7 Estrual cervical mucus properties (pH and fern pattern)

The estrual cervical mucus was collected from buffaloes that has exhibited estrus and collected on glass slide before the insemination. The collected estrual cervical mucus was used to study the following properties.

3.7.1 Hydrogen ion concentration (pH)

The hydrogen ion concentration of cervical mucus was determined by pH indicator papers with 0.5 range difference, immediately after collection. The change of colour of pH indicator paper was matched with standard colour, to determine hydrogen ion concentration (pH).

3.7.2 Fern pattern

Drops of cervical mucus was spread uniformly over a grease free glass slide and air dried. The air dried slide was examined under microscope using low power objective 10X for crystallization pattern of cervical mucus, known as arbonization pattern. The arbonization pattern of observed mucus was grouped as per Galhotra *et al.*, (1971) into typical, atypical and nil type of pattern.

3.8 Pregnancy diagnosis

The pregnancy diagnosis was carried out by per rectal (P/R) examination in inseminated buffaloes after forty five days and confirmed on sixty days. The pregnancy rate was calculated by following formula.

$$\text{Pregnancy rate} = \frac{\text{No. of buffaloes pregnant}}{\text{No. of buffaloes inseminated}} \times 100$$

3.9 Details of drugs used

Following drugs were used during the complete experimental procedure.

3.9.1 Injection Vermact-Vet (Plate 3.2)

Trade name	: Vermact-Vet
Composition	: (Ivermectin IP10mg, Benzyle Alcohol IP 20 mg and Propylene Glycol qs.)
Dose and route of administration	: @ 1 ml per 50 kg body wt s/c
Presentation	: Vermact-Vet is available in pack of 50 ml vial
Manufactured by	: Mankind India Pvt. Ltd.



Plate 3.1. Selection and gynaecological examination of experimental buffalo



Plate 3.2. Inj. Vermact VET used for pre-synchronization treatment

3.9.2 Injection Intavita-H (Plate 3.3)

Trade name	: Intavita-H
Composition	: (Vitamin A 2,50,000 IU, Vitamin D ₃ 25,000 IU, Vitamin E 100 IU, Biotin 12.5 mcg)
Dose and route of administration	: Cattle and Buffalo 5 ml deep intramuscular
Presentation	: Intavita- H is available in pack of 10 ml vial
Manufactured by	: Intas Animal Health Private Limited.

3.9.3 Chelated mineral mixture (Plate 3.4)

Trade name	: Totavit strong (Metho-Chelated) mineral mixture
Composition	: (Cobalt-150 mg, Copper-4.200g, Iodine-350mg, Magnesium- 6.500g, Iron-1.750g, Manganese-1.500g, Potassium-150mg, Selenium-10 mg, Sodium 20mg, Sulphur-9.200g, Fluorine-60 mg, Zinc-9.600g, Calcium-250g, Phosphorus-127.5 g, D.L. Methionine-1.929g, L. Lysine-4.40g, Vitamin A-750000 IU, Vitamin D ₃ -75000 mg, Vitamin E-300mg, Vitamin B ₆ - 20mg, Niacinamide- 1.200g)
Dose, route of administration and ratio	: Cows and Buffalo – 50 g / head / day orally
Presentation	: Totavit strong (Metho-chelated) mineral mixture is Available in pack of 1 kg.
Manufactured by	: Mankind India Pvt. Ltd.

3.9.4 Gonadotropin releasing hormone (GnRH Analogue) injection (Plate 3.5)

Trade name	: Ovulanta
Composition	: Buserelin-Acetate
Dose and route of administration	: 10 µg per animal administered on 0 th & 9 th day intramuscularly
Presentation	: Ovulanta is available in pack of 5 ml (4 mcg/ml)
Manufactured by	: Kilitch Drugs (India) Ltd. Navi Mumbai
Marketed by	: Vet Mankind, Divisoin of Mankind Pharma Ltd

3.9.5 Cloprostenol sodium injection (PGF₂α) (Plate 3.6)

Trade name	: Estrumate
Composition	: Cloprostenol sodium 263 µg/ml equ. To 250 ug cloprostenol
Dose and route of administration	: 500 µg (250 ug/ml) per animal i/m
Presentation	: Estrumate is available in pack of 20 ml
Manufactured by	: Vet Pharma Friesoythe GmbH, Germany.
Marketed by	: Intervet, India Pvt. Ltd.

3.10. Statistical analysis

The statistical analysis was carried out using suitable statistical tests using Web Agri Stats Package (WASP-1) software developed by ICAR, Goa.



Plate 3.3. Inj. Intavita-H used for pre-synchronization treatment

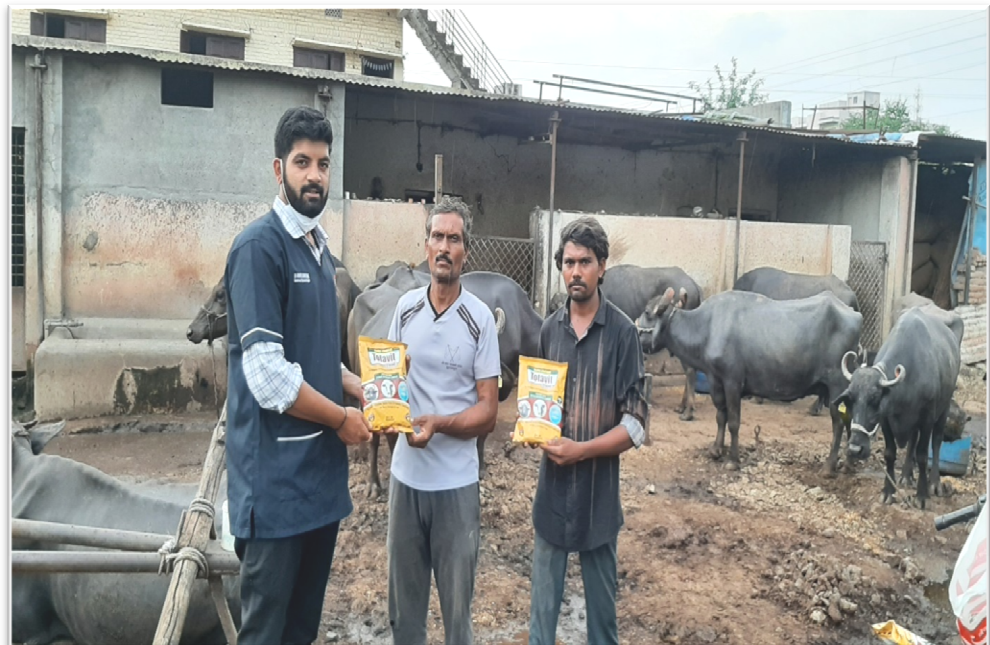


Plate 3.4. Totavita strong mineral mixture supplement to buffalo



Plate 3.5. Inj. Ovulanta used for estrus synchronization



Plate 3.6. Inj.Estrumate used for estrus synchronization in buffalo



Plate 3.7. Artificial insemination in synchronized buffalo



CHAPTER - IV

Results & Discussion

CHAPTER – IV

RESULTS AND DISCUSSION

Estrus synchronization and use sex sorted semen are modern reproductive technologies for maximizing the productivity of dairy herds by production of more females in a herd. Considering this principle, the present study entitled with “Fertility of sexed semen in Ovsynch and double PG Ovsynch protocols in buffaloes” was conducted keeping in view with experimental design described in “Materials and Methods”. Results obtained in present study are presented in tabular forms and discussed under following objectives.

- 1) Efficacy of Ovsynch and double PG Ovsynch protocols in buffaloes
- 2) Estrus signs exhibited in Ovsynch and double PG synchronization protocols in buffaloes
- 3) Estrual cervical mucus properties in synchronized estrus in buffaloes
- 4) Fertility after sex sorted semen in estrus synchronized by Ovsynch and double PG Ovsynch protocols in buffaloes.

4.1 Efficacy of Ovsynch and double PG Ovsynch protocols in buffaloes

In the present research experiment, total twenty four post partum buffaloes were selected and divided randomly into two groups comprising twelve buffaloes in each group. The buffaloes from Group-I were treated with Ovsynch while from Group-II were treated with double PG Ovsynch synchronization protocol. The number of buffaloes responded to the synchronization treatment and exhibited estrus were twelve (100%) in each group, respectively (Table 4.1). The buffaloes showed better treatment response for Ovsynch and double PG Ovsynch synchronization protocol.

Table 4.1 Comparative efficacy of Ovsynch and double PG Ovsynch protocols in buffaloes

Groups	Treatments/ protocol	No. of buffalo synchronized	No. of buffalo heifers exhibited estrus	No. of buffalo responded (%)
Group-I (Ovsynch protocol)	Day- 0 Initial treatment Day- 7 GnRH Day- 14 PGF ₂ α Day- 16 GnRH	12	12	100
Group-II (Double PG Ovsynch protocol)	Day- 0 Initial treatment Day- 7 GnRH Day- 14 PGF ₂ α Day- 15 PGF ₂ α Day- 16 GnRH	12	12	100

The results of present study for estrus exhibition or synchronization after Ovsynch treatment (Group-I) are in agreement with results obtained by Vijayrajan *et al.* (2007), Ghuman *et al.* (2009), Sharma *et al.* (2010) and Nakrani *et al.* (2014), Prasad and Anandrao *et al.*, (2014), Kalaswa *et al.*, (2017) and Savani *et al.*, (2017) reported 100 percent estrus response with Ovsynch synchronization in buffaloes.

The slightly lower estrus response to Ovsynch treatment than present study were reported by Qin *et al.*, (2009) responds 91.7 per cent in Swamp buffaloes, Thorat *et al.*, (2012) and Buhecha *et al.*, (2016) reported 87.50 per cent and 83.33 per cent in anestrus buffaloes, Ingawale *et al.*, (2007) reported 86.66 per cent estrus induction in Murrah buffaloes, Kumar *et al.*, (2010) reported 85.35 per cent estrus exhibition in postpartum buffaloes and Roy and Prakash (2009) reported 82 per cent estrus exhibition in buffaloes. In Ovsynch protocol, Paul and Prakash (2005) and Oropeza *et al.* (2010) reported 90 per cent ovulation in lactating Murrah buffaloes. Hamman *et al.*, (2009) reported 70 per cent in buffalo heifers and Ali *et al.*, (2010) reported 50 per cent in post partum anestrus buffaloes. The research worker Kalwar *et al.*, (2016), and Rathore *et al.*, (2017) reported lower estrus response in buffaloes.

Very lowest efficacy response was recorded by Alyas *et al.*, (2013) and Noui and Boucif (2020) observed 33.00 per cent in Murrah buffaloes.

The results of present study for estrus exhibition or synchronization after double PG Ovsynch treatment (Group-II) are in concurrence with results obtained by Carvalho *et al.*, (2015), Wiltbank *et al.*, (2015), Borchardt *et al.*, (2018), Nowickiv *et al.*, (2019), Rheinberger *et al.*, (2020), Borchardt *et al.*, (2021), Minela *et al.*, (2021) and Tippenhauer *et al.*, (2021) reported 100 percent estrus exhibition and inseminated after double PG Ovsynch synchronization in buffaloes.

The variation in the estrus response could be due to differences in parity, management practices, estrus detection methods, body score of animals, cyclic stage of animals and type and dose of agonist used in the synchronization protocol. The low estrus response as well as ovulation observed by some authors may be due to implementation of the protocol in non breeding season or during summer season. The reproductive seasonality of buffalo due to the melatonin secretion as an endocrinology variation is observed during the year (Zicarelli, 1999).

4.2 Estrus signs exhibited in Ovsynch and double PG Ovsynch protocols

4.2.1 Estrus signs in Ovsynch and double PG Ovsynch protocols

Estrus signs such as tumefaction of vulvar lips, vaginal discharge, and congestion of vaginal mucus membrane were recorded in the Ovsynch (Group-I) and double PG Ovsynch (Group-II) synchronized buffaloes. In Group-I, the tumefaction of vulval lips symptom was shown by 33.33 percent (4/12) buffaloes, congestion of vaginal mucus membrane shown by 50 percent (6/12) buffaloes, signs of vaginal discharge and frequent micturition was shown by 25 (3/12) percent cows. In the Group-II, the tumefaction of vulval lips symptom was shown by 41.66 percent (5/12) buffaloes, congestion of vaginal mucus membrane shown by 58.33 percent (7/12) buffaloes and the signs of vaginal discharge as well as frequent micturation was shown by 33.33 (4/12) percent buffaloes (Table 4.2 and Fig.

4.1). Congestion of vaginal mucus membrane was predominant signs shown by buffaloes in Ovsynch as well as double PG Ovsynch groups.

Table 4.2 Estrus signs exhibited in Ovsynch and double PG Ovsynch synchronization protocols in buffaloes.

Groups	No. of buffaloes exhibited estrus	Estrus signs exhibited by buffaloes			
		Tumification of vulval lips	Congestion of vaginal mucous membrane	Vaginal discharge	Frequent micturation
Group-I (Ovsynch protocol)	12	4 (33.33%)	6 (50.00%)	3 (25.00%)	3 (25.00%)
Group-II (Double PG Ovsynch protocol)	12	5 (41.66%)	7 (58.33%)	4 (33.33%)	4 (33.33%)

In present study, the frequent micturation and congestion of vaginal mucus membrane signs shown were lesser than reported by Kundalkar *et al.*, (2014) and Sawant, S.S. (2021) which might be due to high milk production of buffaloes and due to synchronized estrus.

Stevenson *et al.*, (1996) reported signs of nervousness, mucous discharge and occasional mounting activity in only 3 of 85 cows (3.5 per cent) in synchronized buffaloes with Ovsynch protocol. Neglia *et al.*, (2003) reported that the mucous and estrus behavior were only observed in rare occasion. However, 88 per cent of Italian Mediterranean buffaloes treated with Ovsynch protocol had a tonic uterus on the day of AI. The variation in the estrual signs shown by buffaloes may be due to season, feeding and management of buffaloes, frequency of estrus detection, spontaneous or induced estrus and yield of animals.

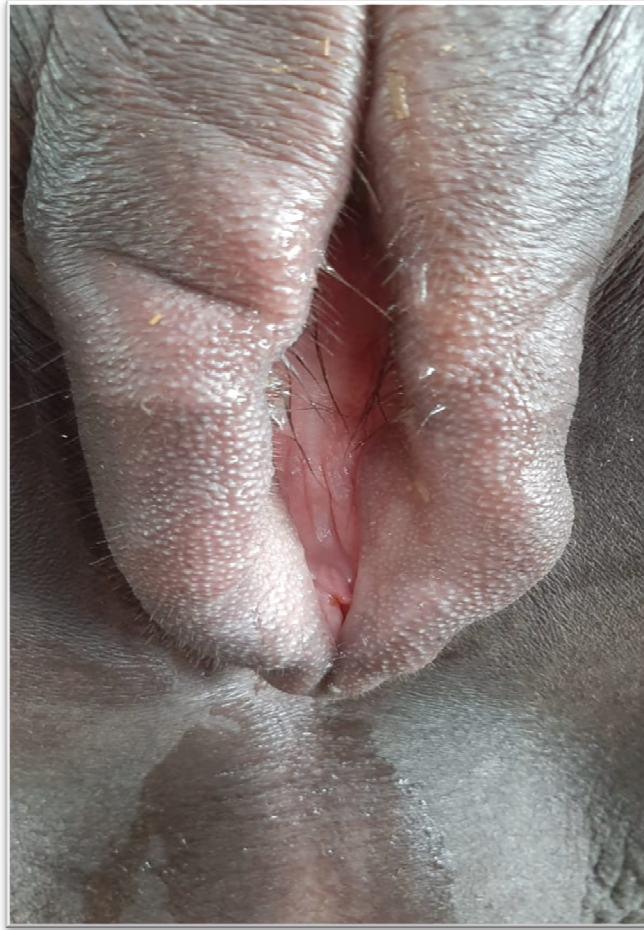


Plate 4.1. Congestion of vaginal mucous membrane in estrus synchronized buffalo

4.3 Estrual cervical mucus properties in synchronized estrus in buffaloes

4.3.1 pH

In the present study, mean pH of cervical mucus is found to be 7.46 ± 0.17 in Group-I and 7.47 ± 0.008 in Group-II. The result is statistically non significant at ($p < 0.01$) level (Table 4.3 and Fig.4.2).

Table 4.3 Average pH in Ovysnch and double PG Ovysnch synchronization protocols in buffaloes

Groups	Average pH
Group-I (Ovysnch protocol)	7.46 ± 0.17^a
Group-II (Double PG Ovysnch protocol)	7.47 ± 0.08^a
Overall Mean	7.465 ± 0.005

Mean with common superscript do not differ significantly from each other at ($p < 0.05$)

The result of present study for pH is in correlation Verma *et al.* (2014) reported cervical mucus of 68.09 per cent estruses were within pH range of 7.5 -8.0 while 19.15 per cent and 12.76 per cent cervical mucus were in pH range of >8 and 7.0-7.5, respectively in Murrah buffaloes. Wanker, M.S. (2016) reported the mean pH of cervical mucus is 7.36 ± 0.12 in Group-I (Clomiphene citrate induced estrus) and 7.53 ± 0.15 in group-II (Ovysnch synchronization protocol) in buffalo heifers which is in harmony with present research findings.

Rao and Rao (1982) observed the mean pH of cervical mucus was 7.93 and appeared to be slightly higher than results of present study. Pattabiraman *et al.*, (1967) recorded the pH of cervical mucus from healthy and regular breeder cow fall within range of 7.0 – 9.0 with the mean of 8.03 ± 1.22 which slightly higher than present findings. It was reported that it tended always to be alkaline and rarely acidic.

Slightly lower pH than the present study was reported by Wani *et al.* (1979), Pandey *et al.* (1983), Singh and Kharche (1984) and Umashanker *et al.* (1984). The variation in the pH value of cervical mucus might be due to mucus collected in different condition like spontaneous estrus, a true induced estrus and in other infertility condition.

4.3.2 Fern pattern

The cervical mucus samples were collected from Group-I and Group-II buffaloes and typical, atypical and nil type of fern pattern was studied. In present study, typical, atypical and nil pattern was observed in 33.33 percent (1/3) buffaloes, respectively in Group-I. In Group-II, typical fern pattern was observed in 50.00 percent (2/4) buffaloes while atypical and nil pattern was observed in 25.00 percent (1/4) buffaloes, respectively. The overall percentage of typical, atypical and nil pattern was 3 (42.85 percent), 2 (28.57 per cent) and 2 (28.57 per cent) in buffaloes (Table 4.4 and Fig 4.3).

Table 4.4 Fern pattern of cervical mucus in Ovsynch and double PG Ovsynch synchronization protocols in buffaloes

Groups	Fern pattern type (%)		
	Typical	Atypical	Nil
Group-I (Ovsynch protocol)	1/3 (33.33)	1/3 (33.33)	1/3 (33.33)
Group-II (Double PG Ovsynch protocol)	2/4 (50.00)	1/4 (25.00)	1/4 (25.00)
Overall Mean	3/7 (42.85)	2/7 (28.57)	2/7 (28.57)

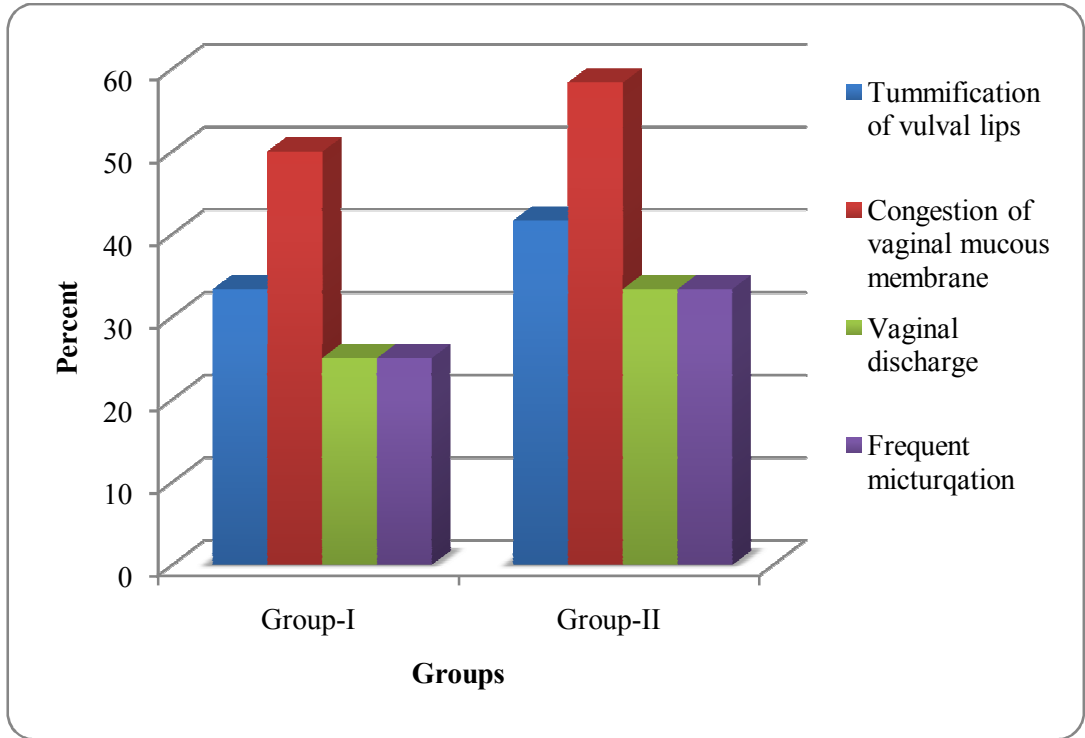


Fig. 4.1. Estrus signs exhibited in Ovsynch and double PG Ovsynch synchronization protocols in buffaloes.

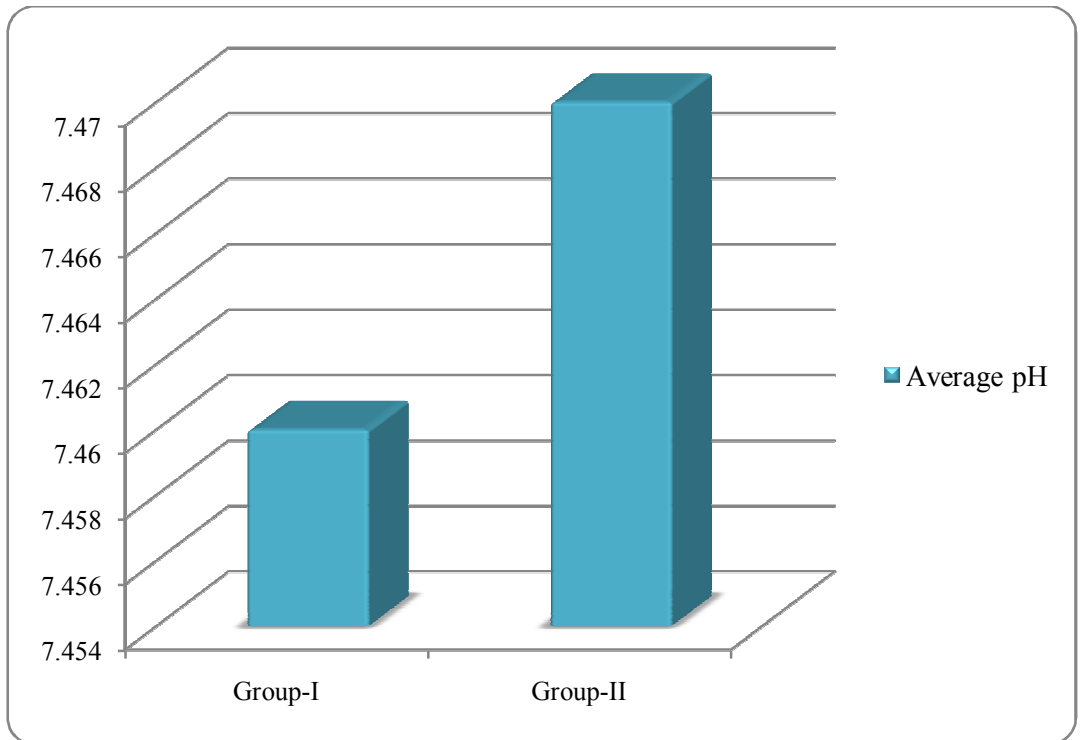


Fig. 4.2 . Average pH in Ovsynch and double PG Ovsynch synchronization protocols in buffaloes.

The present findings are slightly in harmony with Salphale *et al.*, (1993) observed typical (55.56 per cent) and atypical (27.28 per cent) type of fern pattern in estrus synchronized cows.

Shrivastav *et al.*, (2000) observed typical (63.33 per cent) which is higher than present results while atypical (26.67 per cent) and nil (10 per cent) type of fern pattern in cows, respectively in which typical and nil pattern is higher while atypical pattern is slightly lower than result of present study. Varma *et al.*, (2014) observed typical (54.25 per cent), atypical (37.24 per cent) and nil (8.51 per cent) type of arbonization/ fern pattern in Murrah buffaloes, respectively in which typical and atypical fern pattern are higher while nil pattern is lower than present results.

Bishnoi *et al.*, (1982) observed 96 per cent typical crystallization pattern which is higher than the result of present study. Sharma *et al.* (2011) recorded typical, atypical and nil patterns of cervical mucus in normal cyclic buffaloes which is 39.34 per cent, 42.63 per cent and 18.03 per cent, respectively in which typical pattern is lower while atypical pattern is higher than result of present study. Maximum number of buffaloes conceived those with typical pattern (45.83 per cent) and atypical pattern (26.92 per cent).

Wankar, M.S. (2016) observed 6.66 percent (2/3) typical and 3.33 percent (1/3) atypical fern pattern in estrus induced by clomiphene citrate treatment in buffalo heifers while 50.00 percent (5/6), 33.33 percent (2/6) and 16.16 percent (1/6) fern pattern in synchronized estrus with Ovsynch protocol in buffalo heifers.

Typical estrus and the appearance of cervical mucus discharge indicate follicular maturation and increased level of estrogen. Although, fern like patterns are found to be present in mucus during follicular phase and typical fern pattern is indicative of standing estrus. Modi *et al.*, (2011) indicated that typical fern pattern is said to be indicative of ovulatory heat whereas in silent or weak estrus atypical fern pattern is observed which is not concurrence with result of present study.

4.4 Fertility after sex sorted semen in estrus synchronized by Ovsynch and double PG Ovsynch protocols in buffaloes.

The pregnancy rate was 33.33 percent (4/12) in Ovsynch synchronized buffaloes (Group-I) while 41.66 percent (5/12) in double PG Ovsynch synchronized buffaloes (Group-II), respectively. The lower pregnancy rate was observed in Ovsynch synchronized buffaloes compared to double PG Ovsynch synchronized buffaloes, respectively. The results are non significant at ($p < 0.05$) level between the groups (Table 4.5 and Fig. 4.4).

Table 4.5 Pregnancy rate after AI with sex sorted semen in estrus synchronized by Ovsynch and double PG Ovsynch protocol in buffaloes

Groups	No. of buffalo synchronized and inseminated	No. of buffalo became pregnant	Pregnancy rate (%)	Chi square vale
Group-I (Ovsynch protocol)	12	4	33.33 ^{NS}	0.178
Group-II (Double PG Ovsynch protocol)	12	5	41.66 ^{NS}	

NS- Non significant difference for pregnancy rate between the groups ($P < 0.05$)

Pregnancy rate for sex sorted semen in Ovsynch synchronization was in harmony with results reported by Doyle *et al.*, (1999) reported 31.8% pregnancy rate in sex sorted and 40.9% in conventional groups treated with Ovsynch synchronization protocol in HF cows. In conclusion, it was reported insemination with sex-sorted sperm reduced fertility of lactating dairy cows subjected to a TAI program.

Carvalho *et al.*, (2015) reported pregnancy rates per artificial insemination (P/AI) were 32%, 33% and 37 % in Ovsynch, GnRH

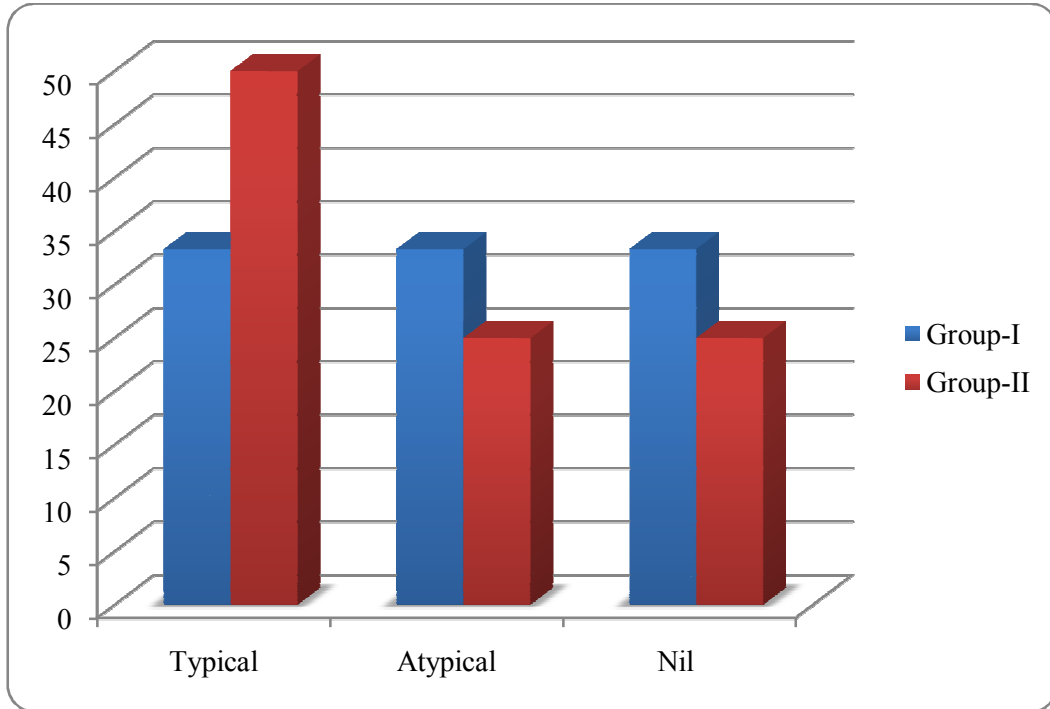


Fig. 4.3 . Fern pattern of cervical mucus in Ovysnch and double PG Ovsynch synchronization protocols in buffaloes.

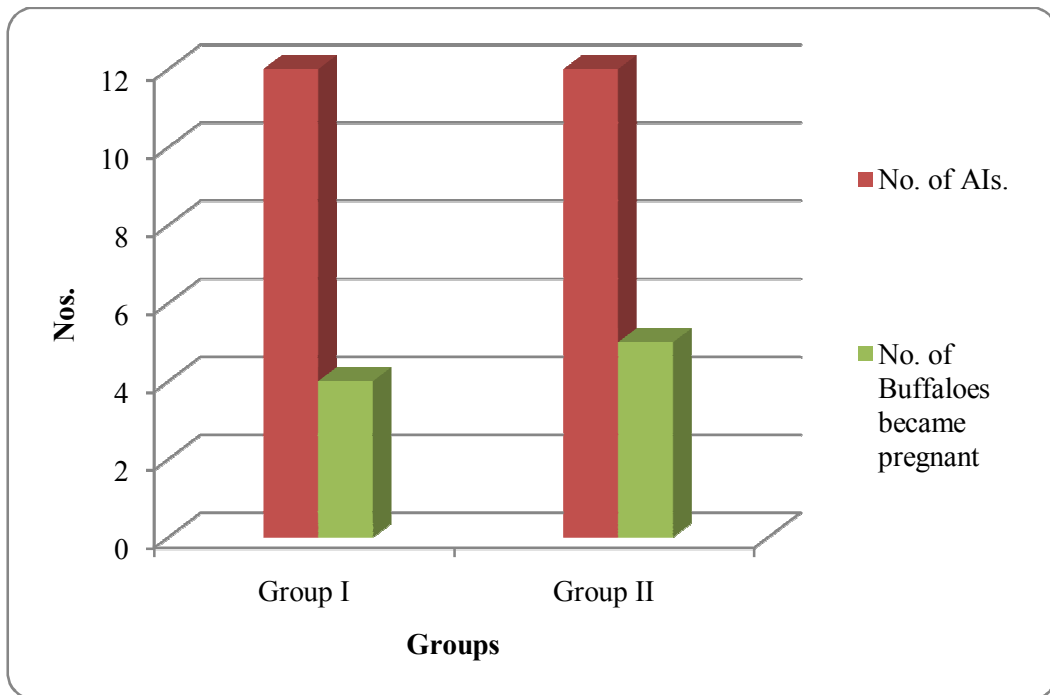


Fig. 4.4 . Pregnancy rate after AI with sex sorted semen in estrus synchronized by Ovysnch and double PG Ovsynch protocol in buffaloes.

presynchronisation treatment before Ovsynch and medicated with Ovsynch and double PG and reported cows treated with double PGF 2α injections and with lower P4 concentration on the day of TAI had better reproductive outcomes than cows which in concurrence with findings observed in present study. Wiltbank *et al.*, (2015) reported increased pregnancies per artificial insemination (P/AI) compared with cows with single dose of PGF (36.1 vs. 33.3%) and observed 9.45% relative increase in P/AI for cows receiving second dose compared with single dose PGF (37.6 vs. 34.4%) and concluded second PGF treatment in Ovsynch-type protocols can increase pregnancy success by about 10%, primarily due to enhanced fertility in multiparous cows which is in accordance with present research findings. Tippenhauer *et al.*, (2021) observed pregnancy per artificial insemination (P/AI) was 31.8% (n=888) in Ovsynch protocol, 33.4% (n=814) in GDPG protocol while 40.3% (n=871) in GPPG protocol, respectively and concluded that addition of second PGF treatment on d 8 during 7 day Ovsynch protocol increased P/AI compared to Ovsynch protocol and to double PGF dose on day 7.

The slightly lower pregnancy rate in two PG treatment during Ovsynch protocol was observed by Nowickiv *et al.*, (2019) reported pregnancy P/AI was 33.3 percent (21/63) in Ovsynch and 31.3 percent (15/48) at 37-40 d gestation in Ovsynch with two treatments of PG 24 hrs apart while 27.00 percent (17/63) and 31.3 percent (15/48) at 70-80 d of gestion. The pregnancy loss rate was higher 6.4 percent (4/63) in Ovsynch group whereas it was nil in double PG treated cows.

Rheinberger *et al.*, (2020) reported 7% more conception rates in Ovsynch with double PG injection than (95% CI 2%-12%) cows receiving the original Ovsynch program and Borchardt *et al.*, (2021) reported there was a clear benefit of an additional PGF 2α treatment during the Ovsynch protocol on P/AI as well as despite additional costs for hormones and labor, an additional treatment with PGF 2α on d 8 was more profitable in 95% of all scenarios because of the associated increase in fertility which is accordance with present result findings.

Minela *et al.*, (2021) reported no evidence was observed of an effect of treatment on pregnancy rate as well as pregnancy losses in Ovsynch, double PG in Ovsynch and double dose of PG in Ovsynch which is not in accordance with results observed in the study.

Morini *et al.*,(2019) reported cumulative PR at day 60 after calving was significantly ($P<0.05$) improved in the group treated with double compared to single PGF2 α treatment (42.9% and 69% for PGF2 and 2PGF2 α cows, respectively and overall pregnancy rate at FTAI was lower during summer compared with winter season (30% vs 41.7%; $P<0.05$) and the pregnancy rate is greater than result of present findings which might be due to use of conventional semen . Sa Filho *et al.*, (2011) reported largest follicle (LF) diameter at TAI and the occurrence of estrus can be used as selection criteria to identify cows with greater odds of pregnancy to receive sex-sorted sperm in TAI programs. In addition, performing TAI with sex- sorted sperm deeper into the uterus did not alter the pregnancy results which is relation with present findings.

The higher conception rate in Ovsynch synchronization was reported than present results by Dawod and Elbaz (2020) revealed that the heifers inseminated with sexed semen had a significantly lower first-service pregnancy rate (51.45%) than those inseminated with conventional semen (61.47%). Heifers achieving puberty before 350 days old had a higher pregnancy rate. Holstein heifers subjected to Ovsynch protocol with sexed semen had an acceptable first-insemination pregnancy rate. The variation may be due to multiparous buffaloes were selected in present study. Sawant, S.S. (2021) reported pregnancy rate was lower after AI with sex sorted semen than conventional semen. The average pregnancy and calving rates for sex sorted semen AIs were 40% and 100% and for conventional semen AIs were 50% and 100%, respectively. The higher pregnancy rate observed might be due to spontaneous estrus than fixed time AI protocols.

Pregnancy rate for sex sorted semen used in spontaneous estrus was in harmony with results reported by Bodmer *et al.*, (2005) observed 33.3% at day 30-40, 27.6% at day 70-90 of pregnancy. Norman *et al.*, (2010) reported 39%; Rhinehart *et al.*, (2011) observed 38% in heifers and 33% in cows. Gaviraghi *et al.*, (2013) reported 31.2%. Healy *et al.*, (2013) and Mallory *et al.*, (2013) observed 31.6% and 38%, respectively. Abdalla *et al.*, (2019) recorded 34% and 32.2% at day 40 and 90, respectively. Karakaya *et al.*, (2014) recorded pregnancy rate at day 31 and at day 62 was 31.8% and 25.7%, respectively and Patel and Jethwa (2019) observed 39.53% pregnancy rate.

In comparison with present research findings recorded, the higher pregnancy rates were recorded for sexed semen in Jersey and Holstein heifer 43% and 46% at dose of 1.5×10^6 and 6×10^6 respectively (Weigal, 2004). Presicce *et al.*, (2005) observed 42.8% at dose of 4×10^6 , Borchersen and Peacock, (2009) reported pregnancy rate in Holstein, Jersey and Red breed was 49.3%, 46.6% and 60.2%, respectively. DeJarnette *et al.*, (2010) observed pregnancy rate 44% and 46% at dose of 2.1×10^6 and 3.5×10^6 . Lu *et al.*, (2010) recorded 69.7%. DeJarnette *et al.*, (2011) observed 44% at dose of 10×10^6 . Gaviraghi *et al.*, (2013) observed 49.8%, 48% and 47% at dose of 4×10^6 , 6×10^6 and 8×10^6 , respectively.

Higher pregnancy rate was also recorded by following researcher during spontaneous estrus in Swamp buffalo 48.5% in Murrah buffalo and 52.5% in Nili-Ravi buffalo by Lu *et al.*, (2015), Djedovic *et al.*, (2016) observed 44%, Kurykin *et al.*, (2016) reported increased pregnancy rate at spontaneous estrus AI (53.4%) than induced estrus AI (41.7%). Shekalgorabi *et al.*, (2017) observed 48.3%, Oikawa, (2019) reported 47.3%. Sharma *et al.*, (2019) observed 49.1% and 42.7% pregnancy rate in cow and buffalo, respectively.

Lower pregnancy rate for sex sorted semen in spontaneous estrus than present research findings were reported by Andersson *et al.*, (2004) recorded 21% at dose of 2×10^6 ; Weigal (2004) observed 31% in Jersey cows. rate in cows.

The variation in the pregnancy rate may be difference in parity (Bodmer *et al.*, 2005), site of semen deposition (Doyle *et al.*, 1999), no. of sperms in frozen semen dose (Seidel *et al.*, 1999; Anderson *et al.*, 2004), AI technician (Weigel, 2004), season (Morini *et al.*, 2019), body condition score, managemental and nutritional conditions, exogenous supplementation of hormones after insemination and spontaneous or induced estrus.



CHAPTER - V

Summary & Conclusions

CHAPTER V

SUMMARY AND CONCLUSIONS

The research experiment entitled “Fertility of sexed semen in Ovsynch and double PG Ovsynch protocols in buffaloes” was conducted at buffalo farms in Akola and at Department of Animal Reproduction, Post Graduate Institute of Veterinary and Animal Sciences, Akola.

The present research work was planned to study comparative efficacy of Ovsynch and double PG Ovsynch synchronization protocols and pregnancy rate with sex sorted semen in both the groups in buffaloes. In present research work, total twenty four buffaloes that have completed post-partum period of forty five days with normal reproductive genitalia and without clinical as well as subclinical infection were selected randomly. The selected multiparous buffaloes were dewormed using injection Ivermectin @ 1 ml per 50 kg body weight subcutaneously and initial treatment with injection vitamin AD₃E&H, 5 ml I/M and supplemented with chelated mineral mixture (1 kg) 50 gm after first gynecological examination. The buffaloes were fed daily greens and dry fodder along with concentrate made of toor churi, wheat bran and cotton seed cakes while fresh water was available ad-lib.

These randomly selected buffaloes were divided into two groups containing twelve buffaloes in each group (n=12). The buffaloes from Group-I was treated with Ovsynch synchronization protocol and from Group-II was treated with double PG Ovsynch synchronization protocol. The buffaloes from the both the groups were inseminated with sex sorted semen (female) after 16-20 hrs after last GnRH injection.

5.1 Efficacy of Ovsynch and double PG Ovsynch protocols in buffaloes.

The number of buffaloes responded to the synchronization treatment and exhibited estrus were twelve (100%) in each group, respectively. The buffaloes showed better treatment response for Ovsynch and double PG Ovsynch synchronization protocol.

5.2 Estrus signs in Ovsynch and double PG synchronization protocols

In Group-I, the tummification of vulval lips symptom was shown by 33.33 percent (4/12) buffaloes, congestion of vaginal mucus membrane shown by 50 percent (6/12) buffaloes, signs of vaginal discharge and frequent micturation was shown by 25 (3/12) percent cows. In the Group-II, the tummification of vulval lips symptom was shown by 41.66 percent (5/12) buffaloes, congestion of vaginal mucus membrane shown by 58.33 percent (7/12) buffaloes and the signs of vaginal discharge as well as frequent micturation was shown by 33.33 (4/12) percent buffaloes. Congestion of vaginal mucus membrane was predominant signs shown by buffaloes in Ovsynch as well as double PG Ovsynch groups.

5.3. Estrual cervical mucus properties in synchronized estrus in buffaloes

5.3.1. pH

The mean pH of cervical mucus is found to be 7.46 ± 0.17 in Group-I and 7.47 ± 0.008 in Group-II. The result is statistically non significant at ($p < 0.05$) level.

5.3.2 Fern pattern

The typical, atypical and nil pattern was observed in 33.33 percent (1/3) buffaloes, respectively in Group-I. In Group-II, typical fern pattern was observed in 50.00 percent (2/4) buffaloes while atypical and nil pattern was observed in 25.00 percent (1/4) buffaloes, respectively. The overall percentage of typical, atypical and nil pattern was 3 (42.85 percent), 2 (28.57 per cent) and 2 (28.57 per cent) in buffaloes.

5.4. Fertility after sex sorted semen in estrus synchronized by Ovsynch and double PG Ovsynch protocols in buffaloes.

The pregnancy rate was 33.33 percent (4/12) in Ovsynch synchronized buffaloes (Group-I) while 41.66 percent (5/12) in double PG Ovsynch synchronized buffaloes (Group-II), respectively. The lower pregnancy rate was observed in Ovsynch synchronized buffaloes compared to double PG

Ovsynch synchronized buffaloes, respectively. The results are non significant at ($p < 0.05$) level between the groups.

Conclusions

1. Ovsynch and double Ovsynch protocols are effective for estrus synchronization in buffaloes.
2. Congestion of vaginal mucus membrane was predominant sign shown by buffaloes in Ovsynch as well as double PG Ovsynch synchronization protocols.
3. The pregnancy rate in double Ovsynch synchronization protocol is higher than Ovsynch protocol. Hence double PG Ovsynch synchronization protocol is used to achieve higher pregnancy rate in sex sorted semen in buffaloes.



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Appendices

APPENDIX – I

pH of cervical mucus in Ovsynch and double PG Ovsynch synchronized buffalo

Sample – 1

No. of Observation	:	3
Average	:	7.467
Standard Deviation	:	0.306
Variance	:	0.093

Sample – 2

No. of Observation	:	4
Average	:	7.475
Standard Deviation	:	0.171
Variance	:	0.029

Test Result

T-Statistic	:	-0.047
T-Table (0.05)	:	2.571
T-Table (0.01)	:	4.032

APPENDIX – II

Pregnancy rate in Ovsynch and double PG Ovsynch synchronized buffalo

Factor M Means

M 1	:	6.000
M 2	:	6.000

Factor N Means

N 1	:	4.500
N 2	:	7.500

Test Result

Chi-Square Statistic	:	0.178
Chi-Square Table (0.05):	:	3.84
Chi-Square Table (0.01):	:	6.64

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Vita

VITA

The author **Dahatonde Akshay Shripati** was born on 6th feb 1997 at his native place Ahmednagar, Ahmednagar district of Maharashtra. He completed her SSC examination in 2012 with first class from Residential Highschool, Ahmednagar and HSC in 2014 with first class from Residential, Junior College, Ahmednagar. Being interested in the field of biological sciences, he joined Krantisingh Nana Patil College of Veterinary and Animal Sciences, Shirwal, Maharashtra and completed the B.V.Sc. and A.H. degree with honours in 2019.

Afterwards joined M.V.Sc degree course in the discipline of Post Graduate Institute of veterinary science, Akola, MAFSU, Maharashtra. he has actively participated in different other extension activities carried out by the Department. During her B.V.Sc and M.V.Sc courses, he participated in different National Programs like National Service scheme (NSS). he also participated in different Conferences, Seminars, Symposia and Training Programme, Workshops from different disciplines like Veterinary Pathology, Veterinary Nutrition, Veterinary Medicine, Wild Life and Veterinary Pharmacology & Toxicology. .



Thesis Abstract

THESIS ABSTRACT

- a) **Title of the thesis (in Capital letters)** : **“FERTILITY OF SEXED SEMEN IN OVSYNCH AND DOUBLE PG OVSYNCH PROTOCOLS IN BUFFALOES”**
- b) **Full name of student** : **DAHATONDE AKSHAY SHRIPATI**
- c) **Name and address of Major advisor** : **Dr. M. V. Ingawale**
Assistant Professor,
Department of Animal Reproduction,
Gynaecology and Obstetrics,
PGIVAS, Akola
- 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 number of pages In the thesis** : 59
- h) **Number of words in the abstract** : 283
- i) **Signature of Student** :
- j) **Signature, Name and address of forwarding authority** :

(C. H. Pawshe)

Head

Department of Animal Reproduction,
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Akola.

ABSTRACT

The research experiment entitled “Fertility of sexed semen in Ovsynch and double PG Ovsynch protocols in buffaloes” was conducted to study efficacy of Ovsynch and double PG Ovsynch synchronization protocols on pregnancy rate in buffaloes. Total twenty four buffaloes were selected randomly and dewormed using pre synchronization medicinal

treatment. The buffaloes from Group-I was treated with Ovsynch synchronization protocol and from Group-II was treated with double PG Ovsynch synchronization protocol. The buffaloes from the both the groups were inseminated with sex sorted semen (female) after 16-20 hrs after last GnRH injection. The number of buffaloes responded to the synchronization treatment and exhibited estrus were twelve (100%) in each group, respectively. Congestion of vaginal mucus membrane was predominant signs shown by buffaloes in Ovsynch as well as double PG Ovsynch groups. The mean pH of cervical mucus is found to be 7.46 ± 0.17 in Group-I and 7.47 ± 0.008 in Group-II. The result is statistically non significant at ($p < 0.05$) level. The typical, atypical and nil pattern was observed in 33.33 percent (1/3) buffaloes, respectively in Group-I. In Group-II, typical fern pattern was observed in 50.00 percent (2/4) buffaloes while atypical and nil pattern was observed in 25.00 percent (1/4) buffaloes, respectively. The pregnancy rate was 33.33 percent (4/12) in Ovsynch synchronized buffaloes (Group-I) while 41.66 percent (5/12) in double PG Ovsynch synchronized buffaloes (Group-II), respectively. The lower pregnancy rate was observed in Ovsynch synchronized buffaloes compared to double PG Ovsynch synchronized buffaloes, respectively. The results are non significant at ($p < 0.05$) level between the groups. The pregnancy rate in double Ovsynch synchronization protocol is higher than Ovsynch protocol. Hence double Ovsynch synchronization protocol is used to achieve higher pregnancy rate in sex sorted semen in buffaloes.

प्रबंध सारांश

प्रबंध सारांश

१. प्रबंधाचे शिर्षक : “म्हशीमध्ये ओव्हीसींच व दोनदा पीजी ओव्हीसिंच प्रोटोकॉलमध्ये लिंगविनिश्चीत वीर्यमात्रेचा प्रजनन क्षमतेवर परिणाम पाहणे”
२. विद्यार्थ्याचे पूर्ण नांव : दहातोंडे अक्षय श्रीपती
३. मुख्य मार्गदर्शकाचे नांव व पत्ता : डॉ. म. वि. इंगवले
सहायक प्राध्यापक,
पशुप्रजनन व प्रसुतीशास्त्र विभाग,
स्नातकोत्तर पशुवैद्यकीय व पशुविज्ञान
संस्था, अकोला
४. प्रदान केली जाणारी पदवी : एम. व्ही. एसस्सी.
५. पदवी प्रदान करण्याचे वर्ष : २०२१
६. मुख्य विषय : पशुजनन शास्त्र
७. प्रबंधामधील एकुण पाने : ५९
८. प्रबंध सारांशामधील एकुण शब्द : २४५
९. विद्यार्थ्याची सही :
१०. प्रबंधक कार्यवाहीस्तव पाठविणाऱ्या अधिकाऱ्याची सही, नाव व पत्ता : (चैतन्य एच. पावशे)
विभाग प्रमुख
पशुजनन शास्त्र विभाग
स्नातकोत्तर पशुवैद्यक व पशुविज्ञान संस्था, अकोला.

सारांश

सदर संशोधनामध्ये “म्हशीमध्ये ओव्हीसींच व दोनदा पीजी ओव्हीसिंच प्रोटोकॉल मध्ये लिंगविनिश्चीत वीर्यमात्रेचा प्रजनन क्षमतेवर परिणाम पाहणे”. यावर संशोधनात्मक अभ्यास करण्यात आला. सदर संशोधनामध्ये २४ म्हशींची

निवड केली आणि जंतनिमुर्लन व माज एकत्रिकरण पुर्वेचे औषधोपचार करण्यात आले. गृप १ मधील म्हशींना ओव्हिसिंच आणि गृप २ मध्ये डबल पीजी ओव्हिसिंच माज एकत्रिकरण प्रोटोकॉलने उपचार करण्यात आले.

दोन्ही गटातील म्हशींना दुसरे जीएनआरएच इंजेक्शन नंतर १६-२० तासानंतर लिंगविनिश्चीत (मादी) वीर्यमात्रेद्वारे कृत्रिम रेतन करण्यात आले. माज एकत्रिकरण उपचारानंतर प्रत्येक गटात अनुक्रम १२ (१००%) म्हशींनी माज दाखविला. योनी श्लेष्म पडद्याची रक्ताधिक्य ही ओव्हिसिंच म्हशींमध्ये तसेच डबल पीजी गटामध्ये मुख्यत्वे दिसून आली. गर्भाशयाच्या श्लेष्मचा सामू गट १ मध्ये ७.४६ ± ०.१७ आणि गट २ मध्ये ७.४७ ± ०.००८ असल्याचे आढळून आले. याचा परिणाम सांख्यिकी दृष्ट्या (पी <०.०५) पातळीवर फरक लक्षणीय नव्हता. गट १ मध्ये ३३.३३% (१/३) म्हशींमध्ये सामान्य, असामान्य आणि शून्य फर्न नमुना दिसून आला. गट -२ मध्ये सामान्य फर्न पॅटर्न अनुक्रमे ५० (२/४) म्हशी मध्ये दिसला तर असामान्य आणि शून्य नमुना अनुक्रमे २५% (१/४) म्हशींमध्ये दिसून आला. गर्भधारणा प्रमाण ओव्हिसिंच (गट -१) मध्ये ३३% (४/१२) होते. तर डबल पीजी ओव्हिसिंच म्हशींमध्ये (गट -२) ४१.६६% (५/१२) होते. अनुक्रमे डबल पीजी ओव्हिसिंच म्हशींच्या तुलनेत ओव्हिसिंच माज एकत्रिकरण म्हशींमध्ये गर्भधारणेचे प्रमाण कमी दिसून आले. गटातील (पी <०.०५) पातळीवर फरक लक्षणीय नाहीत. डबल पीजी माज एकत्रिकरण मधील गर्भधारणेचे प्रमाण ओव्हिसिंच प्रोटोकॉल पेक्षा जास्त आहे. म्हणून म्हशींमध्ये लिंगविनिश्चीत वीर्य मात्रेद्वारे मध्ये गर्भधारणेचे प्रमाण जास्त साध्य करण्यासाठी डबल पीजी ओव्हिसिंच माज एकत्रिकरण प्रोटोकॉलचा वापर करावा.