FERTILITY EVALUATION AFTER CLOMIPHENE CITRATE AND OVSYNCH PROTOCOL TREATMENT IN BUFFALO HEIFERS

T H E S I S

Submitted
In partial fulfillment of the requirements for the Degree of

MASTER OF VETERINARY SCIENCE
IN
ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS

BY
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(INDIA)
2016

DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled **"FERTILITY EVALUATION AFTER CLOMIPHENE CITRATE AND OVSYNCH PROTOCOL TREATMENT IN BUFFALO HEIFERS"** 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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CERTIFICATE

This is to certify that the thesis entitled **"FERTILITY EVALUATION AFTER CLOMIPHENE CITRATE AND OVSYNCH PROTOCOL TREATMENT IN BUFFALO HEIFERS"** submitted by **WANKAR MEGHA SHRIDHARRAO** to the Maharashtra Animal and Fishery Sciences University, Nagpur in partial fulfillment of the requirement for the degree of **MASTER OF VETERINARY SCIENCE** in **ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS** has been approved by the student's advisory committee after examination in collaboration with the External Examiner.

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ACKNOWLEDGEMENT

This thesis is dedicated in Memory of my beloved father and mother.

Acknowledgement is not just an formality to express thank but the words lacks to express my feeling to all who helped me to mould this research work, without their help I cannot have put up my thesis. Innumerable people have assisted me and guided me through out my journey

I take this opportunity to express deep sense of heartiest gratitude and sincere regards with respect to my honorable guide and Chairman of my advisory committee Dr. M. V. Ingawale, M.V.Sc., Ph.D. (NET), Assistant Professor, Department of Animal Reproduction, Gynaecology and Obstetrics, Post Graduate Institute of Veterinary and Animal Sciences, Akola, (MAFSU, Nagpur). It is my proud privilege to offer sincere and well devoted thanks to him for his worthy guidance, valuable suggestions, constant encouragement, gentle and caring attitude not only throughout period of my research work but also throughout my post graduation. I forever will be thankful to have this opportunity to work under his guidance.

I express my cordial thanks to Dr. H. S. Birade, M.V.Sc., Ph.D., Associate Dean and Head, Department of Animal Reproduction, Gynaecology and Obstetrics, Post Graduate Institute of Veterinary and Animal Sciences, Akola for his valuable suggestions and parental affection and for providing all necessary facilities for the completion of this research work.

I also express my extreme thanks to Dr. S. G. Deshmukh, M.V.Sc., Assistant Professor of Animal Reproduction, Gynaecology and Obstetrics, Post Graduate Institute of Veterinary and Animal Sciences, Akola.

It gives me an immense pleasure to place on record the deepest sense of gratitude to Dr. S. W. Hajare, M.V.Sc., Ph.D, Assistant Professor, Department of Veterinary Pharmacology and Toxicology, Post Graduate Institute of Veterinary and Animal Sciences, Akola for his kind support and inspiring guidance throughout the course of present investigation.

I am also thankful to Dr. R. S. Ingole, M.V.Sc., Assistant Professor, Department of Pathology, Post Graduate Institute of Veterinary and Animal Sciences, Akola for moral support, valuable guidance and kind help.

It gives me an immense pleasure to express my gratitude and sincere regards to Dr. M.F.M.F. Siddhiqui, M.V.Sc. Assistant Professor, Department of Medicine, Ethics and Jurisprudence, PGIVAS, Akola for his valuable guidance and kind help.

I am also thankful to Dr. R. V. Raulkar, M.V.Sc. Assistant Professor, Department of Veterinary Surgery and Radiology, PGIVAS, Akola for his valuable guidance and kind help.

I render my sincere thanks to Dr. Sajit Ali, Assistant Professor, Department of Animal Genetics and Breeding, PGIVAS, Akola for his valuable guidance.

I am also thankful to Dr. M. G. Thorat, M.V.Sc., Ph.D. Associate Professor and Head, Department of Veterinary Surgery and Radiology, PGIVAS, Akola for his valuable guidance.

I take this opportunity to express my sincere thanks to Dr. S. P. Waghamare, M.V.Sc., Ph.D., Hospital Superintendent, T.V.C.C., Akola and Dr. K. S. Pajai, Assistant Professor, Department of Medicine, Ethics and Jurisprudence, PGIVAS, Akola for his valuable guidance and kind help.

It gives me an immense pleasure to express my gratitude and sincere regards to Dr. P. S. Kuralkar, Assistant Professor, Department of Animal physiology, PGIVAS, Akola for her valuable guidance and kind help.

It gives me an immense pleasure to express my gratitude and sincere regards to Dr. Nichal, Livestock Development Officer, Veterinary Dispensary, Kurankhed for his moral support and valuable co-operation.

I wish to thank my dearest father Late. Shri. Shridharrao Wankar and my mother Mrs. Prabha Wankar if not for their hard work and support I wouldn't have achieved what I have today. I take this opportunity to dedicate this work to them.

It is like a drop in the ocean of words that can never reach its mark to acknowledgement infinite love, blessings, sacrifices and constant encouragement of my beloved parents, my husband Mr. Manoj Sarode and my daughters Mrunal and Manjiri Sarode, my brothers Mr. Atul Wankar, Mr. Suhas Wankar and my sister Mrs. Varsha Deshmukh who are the whole and sole of my life. They are the backbone of everything I achieved up to and after. Hence, it would be just impossible to get relieved from their bondage. I am also grateful to express my feeling to my mother-in-law Mrs. Durga Sarode and father-in-law Mr. Vasantrao Sarode for their love, blessing and moral support.

I express my regards towards my friends Akshay Bind and Syed Anwar Ali for their constant co-operation not only throughout my research work but also throughout post graduation. I am also thankful to Vaijanath Kale and my junior friends Priyanka Hirole, Vishnu Dalvi, Karthik Iyer for their co-operation. I am also thankful to my friends Roshani Bhoyar, Shubhangi Mitkari, Suvarna Sonwane, Salim Tadvi, Pranjali Pore, Vaishali Wankhede, Arpana Sontakke, Prajyot Dakhane and Mohhamad Ali and Kalpana Pawar for their co-operation.

I am also thankful to Shri. R. N. Adhau, Shri. Kulvant and Shri. P. D. Patil and Shri. Bhaltilak for their help during my research work.

I am very much thankful to Mr. Nikhil Kathiwale (M/s. Nikhil Grafix, Akola) for skilled typing of the manuscript within time.

Last but not least, I thank the entire individual who have in any way been associated with the completion of this work but have not been mentioned so far. My essence thanks to Almighty God who made everything possible.

Place: Akola **(Wankar Megha Shridharrao)**
Date :

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LIST OF ABBREVIATIONS

Abbreviation		Full form
%	-	Per cent
*	-	P<0.05
**	-	P<0.01
/	-	Per
@	-	At the rate of
<	-	Less than
>	-	More than
±	-	Plus Minus
℃	-	Degree Centigrade/ Celsius
µg	-	Microgram
A. I.	-	Artificial insemination
A.M./a.m.	-	At meridium
CD	-	Critical difference
cm	-	Centimeter
DLC	-	Differential Leukocytes Count
et al.	-	Et alia (and others)
etc.	-	Etcetera
Fig.	-	Figure
g%	-	Gram percent/ Gram per deciliter
g/dl	-	Gram per deciliter
GDP.	-	Gross Domestic Product
gm/dl or g/dl	-	Gram
GnRH	-	Gonadotropin Releasing Hormone
GVA	-	Gross Value of output in Agriculture
h/hrs	-	Hours
Hb	-	Hemoglobin
i.e.	-	That is/ id est
im	-	Intra muscular
Inj.	-	Injection
IU	-	International Unit
Kg	-	Kilogram
Ltd.	-	Limited
M.S.	-	Mean Square
mg	-	Milligram
mg/dl	-	Milligram per deciliter
Min.	-	Minute
ml	-	Mililiter
mm	-	Milimeter
N.P.	-	Non-Pregnant
no./No.	-	Number
NS	-	Non-significant
P.M./p.m.	-	Post meridium
PCV	-	Packed Cell Volume
$PGF_2\alpha$	-	Prostaglandin F_2 alpha
S.E.	-	Standard error
S/c	-	Subcutaneous
Sr.No.	-	Serial Numbers
viz.	-	Videlicet
Vs/vs.	-	Versus

CHAPTER I

INTRODUCTION

The dairy and livestock sector plays a very important role in national economy of India by contributing close to one third of gross income of rural households and nearly half of gross income in case of those without land (Bhasin, 2016).

The contribution of livestock sector in national economy in terms of GDP was 3.9% during 2013-2014. As per the report of National Accounts Statistics, 2015 the percentage contribution of livestock in terms of gross value of output (GVA) in agriculture was 32.91%. India possesses 108.702 million buffaloes and contributes around 21.23% of the total livestock population as well as 51 % of total milk production (19th Livestock census). So buffalo is named as “Black Gold” has a pivotal and pre-eminent importance in the livestock sector of the country.

Reproduction is the back bone of animal production. Breeding of buffaloes throughout the year plays a vital role for maintaining dairy as viable unit. Inherent reproductive problems like delayed puberty, higher age at first calving, post partum anoestrus, silent heat, seasonality in breeding and low conception rate limit the productivity of buffalo (Terzano *et al.,* 2012). The anoestrus, poor estrus symptoms, erratic duration of estrus and low conception rate are encountered usually in buffalo heifers. The delay in conception is one of the constraints that cause the low reproductive efficiency not only hampered the net calf crop but also life time milk production leading to basic economic losses to buffalo owners. Out of these constraints, anoestrus particularly summer anoestrus is major infertility problem showing suspension of sexual activity with inactive smooth ovaries (Qureshi *et al.,* 1999) and abnormal hormonal profile (Razdan, 1988). The incidence of summer anoestrus varies between 36.6 and 59.5 % (Luktuke and Sharma, 1978; Singh *et al.,* 1989).

The different treatment regimes like herbal heat inducer drugs (Mohanty *et al.,* 2007), biostimulation (Ahmed *et al.,* 2010), vitamin and mineral mixture supplementation (Mathur *et al.,* 2005) and hormonal

preparations (Mavi *et al.,* 2007, Sirmour *et al.,* 2006) are practiced to induce estrus. Out of these, clomiphene citrate treatment orally (Kadu and Chede, 1992, Purohit and Bishnoi, 1993) is most convenient and effective treatment practiced by dairy owners whereas, the Ovsynch is synchronization of ovulation protocol widely utilized by veterinarian (Paul and Prakash, 2005; Singh, 2014).

Clomiphene citrate, which belongs to be a group of drugs known as selective estrogen receptor modulators (SERM), is most commonly prescribed drug to treat fertility disorders due to ovulation failure in women (Plouffe, 2000). Clomiphene citrate, a non-hormonal substance with the properties of inducing ovarian activity and promoting ovulation by feedback effects on pituitary gland have been successfully used in domestic animals to induce ovulation and conception (Deshpande *et al.,* 1976 and Hukeri *et al.,* 1979). Clomiphene inhibits estrogen receptors in hypothalamus inhibiting negative feedback of estrogen on gonadotropin release leading to up regulation of hypothalamic-pituitory-gonadal axis. Since estrogen no longer effectively exert negative feedback on hypothamus, GnRH secretion becomes more rapidly pulsatile which results in increased pituitary gonadotropin (FSH and LH) release. After administration of clomiphene citrate, FSH level rises steadily resulting in to the development of new follicles. Those follicles in turn produce estrogen which circulates in the blood and thus the onset of estrus takes place. The efficacy of clomiphene citrate for improving fertility in anoestrus condition with inactive and smooth ovaries was reported by (Reddy *et al.,* 1990; Kankal *et al.,* 2008, Ingawale *et al.,* 2011; More, 2012) in cows and buffaloes.

Ovsynch synchronization protocol consists of combination of GnRH and prostaglandin. Ovsynch is one of the estrus synchronization protocol consisting of two injections of gonadotropic releasing hormone (GnRH) combined with single administration of prostaglandin $F_2\alpha$ ($PGF_2\alpha$) and used in cyclic buffaloes for synchronization of ovulation and fixed time artificial insemination (Singh, 2014). Efficacy of the Ovsynch is dependent on the stage of follicular development at the time of initial GnRH injection (Renesis *et al.,* 2005). The main advantage of Ovsynch protocol is estrus synchronization that 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 Praksh, 2005; Ghuman *et al.,* 2008) in Murrah buffalo heifers (Roy and Prakash, 2009) as well as during non breeding season (Karen and Darwish, 2010).

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 (arbonisation) 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. Therefore may be used in determining stage of estrus for fixing optimum time of insemination in farm animals. The occurrence of crystallization is common to all types of mucus. But degree of crystallization or arbonosation 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 dimishes 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 and buffaloes (Alena *et al.,* 2008).

Hematological and biochemical profile plays a key role in diagnosis of array of different productive and reproductive disorders in different livestock species. The erythrocytic indices diagnosed the general health status and diseases like anemia and its type while the platelet count point to clotting ability. The leucocytic indices provide indication of infection and the probable type of infection. Low hemoglobin level can influence the tissue oxygenation of reproductive tract and can influence cyclicity. Reduced hemoglobin concentrations were reported in anoestrus buffaloes as compared to normal cyclic buffaloes (Kumar *et al.,* 1991). The blood picture may vary in normal cycling and anoestrus animals.

Normal levels of biochemical constituents are of utmost importance for maintaining the functional integrity of the reproductive system (Niazi *et al.,* 2003). Changes in blood biochemical constituents are important indicators of physiological state (Perveen and Usmani, 1993) as well as the reproductive performance of animals (Dutta *et al.,* 1988, Prabha *et al.,* 2000). Arthur (1975) reported that pituitary function may be particularly influenced by blood glucose level. Blood glucose level gives an indication of carbohydrate status and the hypoglycemic herds pose the problem of negative energy balance and suffer from anoestrum in particular during winter. Cholesterol acts as precursor of steroid hormones and its level can indicate circulatory adequence of hormones responsible for normal estrus (Ramkrishna, 1997). Burle *et al.* (1995) reported lowest serum concentration of cholesterol in anoestrus than in cycling cows.

Low level of serum protein may lead to deficiency of certain amino acids that are required for gonadotropin synthesis thereby impairing reproduction (Vohra *et al.,* 1995). El-Azab *et al.* (1993) reported significantly higher serum protein in cyclic cows than the non-cycling one. Blood urea nitrogen (BUN) is the basic component of protein metabolism. The high level of blood urea which has a toxic effect on sperm as well as on the ova that interferes with fertilization and also on developing embryo. The balance of hormone may be altered progesterone levels are low when blood level contains high level of urea. This low progesterone causes early embryonic mortality and reduced conception. The current scientific evidence suggests that it has the largest effect in the embryonic development stage. BUN level

above 20 mg/dl had conception rates under 25 per cent in one of the studies (Ferguson and Chalupa, 1989).

Keeping in view the major problem of summer anoestrus in buffalo heifers which are maintained at field level and use of clomiphene citrate and Ovsynch protocol in anoestrus with importance of cervical mucus properties and hemato-biochemical values for impairing fertility, the present research is planned to study fertility evaluation after clomiphene citrate and Ovsynch protocol treatment in buffalo heifers under field condition during non-breeding season with following objectives.

Objectives

1) To study the efficacy of Clomiphene citrate and Ovsynch protocol for estrus induction in buffalo heifers.
2) To record the time requirement for exhibition of estrus in buffalo heifers.
3) To study the estrual cervical mucus properties in buffalo heifers.
4) To assess the hematological and biochemical parameters in buffalo heifers.
5) To study the first service conception rate in buffalo heifers.

CHAPTER II

REVIEW OF LITERATURE

2.1 Efficacy of Clomiphene Citrate on Fertility

Clomiphene citrate, non-hormonal substance when administered orally acts on hypothalamo-pituitory axis to release GnRH which stimulates the anterior pituitary to secrete luteinizing hormone and follicle stimulating hormone. This also have regulatory effect on peripheral circulation of estrogen and hence pin- point ovulation takes place.

Deshpande *et al.* (1976) studied the efficacy of Fertivet (clomiphene citrate) for induction of estrus in anoestrus cows and buffaloes. The clomiphene citrate was administered 300 mg orally for five consecutive days. Fifty per cent of cows were exhibited estrus within 4 to11days whereas out of nine buffaloes of Murrah breed, 100 per cent of buffaloes were expressed heat within 2 to 8 days of treatment, respectively.

Kaikani *et al.* (1977) studied efficacy of Fertivet (clomiphene citrate) for induction of estrus in anoestrus cows during summer season. Total 30 cows were divided into 3 groups comprising 10 cows in each group. Fertivet was administered as single dose treatment 500 mg to the cows from group A while cows from group B were administered with 300 mg clomiphene citrate for five days. Cows from group C were kept as control without treatment. Fertivet single dose treatment (FVT 500 mg) was found having only superficial effect of congestion of genitalia but there was neither follicular growth nor ovarian activity. 'Fertivet' five-day treatment (FVT 300 mg) gave most encouraging results as 60 percent cases manifested sign of heats.

Hukeri *et al.* (1979) studied 47 Murrah anoestrus lactating buffaloes which had smooth inactive ovaries and not exhibited estrus even six month after calving were selected and divided into two groups. In group I, 35 buffaloes were treated with Fertivet (FVT-300 mg) tablet dissolved in 500 ml of water for a period of 5 days and 12 buffaloes were kept in control group. Number of buffaloes exhibited estrus was 30 (85.72 per cent) and 6 (50.00 per cent) while days were required to induce estrus were 11.13 and 32.83 in group I and group II, respectively. Out of 30 buffaloes exhibiting estrus, 24 (80

per cent) found pregnant in group I and 3 (50 per cent) buffaloes were conceived from control group.

Dugwekar *et al.* (1980) treated 5 Brown Swiss cows, 5 Jersey cross breds cows and 6 Sahiwal anoestrus cows with Fertivet (300 mg) orally for five days. Out of these, 3 Brown Swiss, 3 Sahiwal and 4 Jersey cross breeds exhibited heat within 0-5 days, one Brown Swiss as well as Sahiwal exhibited estrus within 6 -10 days cow and 11-15 days after end of treatment, respectively. Four Brown Swiss, 4 Jersey cross breed were found pregnant with induced estrus while 3 Sahiwal cows conceived with subsequent estrus.

Varma and Kharche (1983) studied comparative efficacy of Fertivet and Lugol' iodine in anoestrus Murrah buffaloes. Buffaloes which has not exhibited estrus 90 days or more post partum were selected and divided into three groups. Group I consisted of 8 anoestrus buffaloes in which 2% Lugol's iodine was painted and infused in cervix. Group II consisted of 5 anoestrus buffaloes which were administered one tablet of Fertivet and received 125 ml of 1% copper sulphate solution orally for five consecutive days. Group III consisted of 8 buffaloes which were kept as control. Experiment was carried out in breeding season and heat detection was done twice daily by vasectomized buffalo bull. Number of buffaloes exhibited estrus was 4 (50 per cent), 3 (60 per cent) and 1(12.50 per cent) while average interval between treatment and induction was 18.75, 21 and 45 days in Group I, Group II and Group III ,respectively. Number of buffaloes conceived were 3 (75 per cent), 3 (100 per cent) and 0 in Group I, II and III, respectively.

Kurien and Madhavan (1985) studied the efficacy of clomiphene citrate for induction of estrus in cows and heifers. Total 28 cows which failed to exhibit estrus even after 60 days postpartum and 41 heifers which failed to exhibit estrus even after 24 months were selected. Nineteen cows and thirty-three heifers were administered 125 ml of 1% copper sulfate solution followed by Fertivet 300 mg dissolved in 300ml water. Nine cows and eight heifers were kept as control and given only 125 ml of 1% copper sulphate solution for 5 days. The ovulatory heat was induced in 13 (68.42 per cent) and 21(63.64 per cent) cows and heifers, respectively treated with FVT-300 while 3 (33.33 per cent) and 3 (37.50 per cent) in control cows and heifers, respectively. The average interval for induction of estrus was 4.57±0.16 days and 8 (42.11 per cent) cows found to be pregnant while

4.48±0.21 days required for induction of heat in heifers with 10 (30.30 per cent) found pregnant, respectively treated with FVT-300. While in control group, 22±4.36 days and 5±0.58 days required for heat induction in cows and heifers, respectively and only 1 (11.11 per cent) found pregnant.

Deen and Tanwar (1988) studied efficacy of clomiphene citrate in seasonal anoestrus buffaloes. Twelve seasonally anoestrus buffaloes were selected and divided into two groups. Six buffaloes from treatment group were administered with clomiphene citrate 300 mg for 5 consecutive days while 6 were kept as control. Four (66.66 per cent) out of six buffaloes ovulated after treatment with clomiphene citrate as compared to none (0 per cent) in control group during summer season. Out of 4, 1(25 per cent) buffalo found pregnant.

Banerjee and Roychaudhary (1989) studied the efficacy of clomiphene citrate on 20 true anoestrus Murrah graded buffaloes. The buffaloes were treated with one tablet of Fertivet dissolved in 300 ml water for 5 consecutive days. The buffaloes which have exhibited estrus were given natural service at induction of estrus. Out of 20 buffaloes, 16 (80 per cent) of buffaloes exhibited estrus with a mean interval of five days for estrus induction and 12 (75 per cent) were conceived.

Reddy *et al.* (1990) studied effect of Clofert-Vet (clomiphene citrate) on postpartum anoestrus crossbred cows and Murrah buffaloes. Twenty each of cross bred cows and buffaloes were administered with125 ml of 10% sodium bicarbonate solution followed by one tablet of Clofert-Vet dissolved in 500ml of water daily for five consecutive days. Ten each of crossbred cows and Murrah buffaloes were given daily with125 ml of 10% sodium bicarbonate solution daily for five consecutive days were kept as control animals. Out of 20 treated cows and buffaloes,12 (60 per cent) crossbred cows and 17 (85 per cent) buffaloes exhibited ovulatoy estrus with an average interval of 8.42 ± 0.98 days and 6.01 ± 0.41 days, respectively. In control group, only 3 (30 per cent) cows and 4 (40 per cent) buffaloes exhibited estrus with an average interval of 30.33 ± 3.39 and 26.00 ± 2.35 days, respectively. Overall conception rate was 55 per cent and 80 per cent in cross berd cows and buffaloes in treatment group and 30 and 40 per cent in control cows and buffaloes.

Kadu and Chede (1992) studied the efficacy of Fertivet (FVT 300) for induction of estrus in 19 true anoestrus buffaloes during summer. The buffaloes were treated with Fertivet (FVT-300) one tablet per day for five days after a drench of 100 ml of 1% copper sulphate solution. Heat detection was carried out by parading aproned buffalo bull. Buffaloes in estrus were bred by A.I. with frozen semen. Out of 19 buffaloes treated with Fertivet, 13 (68.42 per cent) exhibited estrus of which 8(61.53 per cent) conceived to first service, while 3 (15.3 per cent) conceived to second service with overall conception rate was 84.10 per cent.

Purohit and Bishnoi (1993) studied the efficacy of Fertivet (clomiphene citrate) for induction of estrus in which 7 post partum anoestrus Rathi cows and 12 anoestrus Rathi heifers having smooth inactive ovaries and free from genital infection. The selected animals were treated with Fertivet (FVT-300 mg) dissolved in 250 ml of water on first day and on subsequent days, tablet was mixed with jaggery for 4 days. Out of seven, four (57.10 per cent) cows and 10 (83.33 per cent) heifers exhibited estrus with mean time interval of 8.75 days and 5.9 days to induce estrus after completion of treatment in cows and heifers, respectively. The first service pregnancy rate was 50 per cent in cows and 50 per cent became pregnant in third service whereas 71.42 per cent and 28.57 per cent heifers conceived in first and second service, respectively.

Reddy *et al*. (1994) studied the efficacy of Fertivet (clomiphene citrate) to induce estrus in post-partum anoestrus buffaloes more than 180 days post-partum. In this experiment, total 20 anoestrus buffaloes were given one tablet of Fertivet administered orally dissolved in 500 ml of distilled water daily for 5 consecutive days with prior drenching of 125 ml of 1% copper sulphate solution while the control group buffaloes were not received any treatment (n=20). Estrus induction was observed in 16 (80 per cent) buffaloes with a mean interval of 21 days and 6 (30 per cent) buffaloes with a mean interval of 30.16 days in treated and control group, respectively. Out of sixteen, 14 (87.50 per cent) and out of six, 4(66.66 per cent) buffaloes were conceived in Fertivet treated and control group, respectively.

Kankal *et al*. (2008) selected 12 Red Kandhari and 18 Deoni cows which have not exhibited heat 90 days postpartum and having smooth and inactive gonads were divided into four groups. In group I, six Red

Kandhari anoestrus cows were treated with tablet Fertivet (clomiphene citrate) 300 mg orally with 125 ml of 1% copper sulphate solution prior to administration of Fertivet daily for 5 days. In group II, six Deoni anoestrus cows were treated with tablet Fertivet (clomiphene citrate) 300 mg orally daily for 5 days and six Deoni cows were kept as untreated control. In group I, three (50 per cent) cows exhibited estrus on the day 2 and two on day 5. In group II, out of six, three (50 per cent) cows exhibited estrus two on day 5 and one on day 6. All the six cows from untreated control group did not exhibit any sign of estrus.

Ingawale *et al.* (2011) studied the efficacy of clomiphene citrate on fertility potential in buffaloes. Total twenty one non-descript buffaloes more than seven month postpartum anoestrus were selected and treated with injection Ivermectine @ 1ml per 50 kg body weight s/c, injection vitamin AD_3k 5ml I/M, Injection of Phosphorus @ 10 ml I/M and chelated mineral mixture (Agrimin Forte) 50 gm daily for 10 days. After 10 day, daily one tablet Ovulanta (clomiphene citrate) was given for five days. Out of 21 buffaloes, 8 (38.09 per cent) buffaloes exhibited estrus and inseminated. Out of eigh inseminated buffaloes, 6 (75 per cent) found pregnant.

More (2012) studied the efficacy of clomiphene citrate in true anoestrus crossbred cows. In this study eight cows were treated with bolus of Fertifin (clomiphene citrate) 300 mg orally per day for five days. The dose of clomiphene citrate was repeated on the day tenth in cows which were not responding to first dose whereas eight cows were left untreated and kept as control. In treatment group out of eight, 4 (50 per cent) cows exhibited estrus while mean time interval of 7.67 days (184.04 hrs) for induction of estrus after first dose of clomiphene citrate and one out of remaining cows (25 per cent) exhibited estrus while mean time interval of 9.75 days (234.00 hrs) for induction of estrus after second dose. In treatment group out of five, three cows found pregnant. In control group, out of eight, one (12.50 per cent) cow exhibited estrus at interval of 29 days and remain pregnant (100 per cent).

2.2 Efficacy of Ovsynch Protocol on Fertility

Baruselli *et al*. (2001) synchronized two hundred 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 $PGF_2\alpha$, 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 $PGF_2\alpha$ 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 per cent (62/132) and 50 per cent (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 $PGF_2\alpha$ (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 $PGF_2\alpha$ on day 7 and 12.5 mg Luprotial-lutropin-v on day 9. The conception rate was 56.5 per cent and 64.2 per cent 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 $PGF_2\alpha$ 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 per cent.

Neglia *et al.* (2003) synchronized one hundred eleven Italian Mediterranean buffalo cows with 100 ug GnRH on day 0, 375 ug $PGF_2\alpha$ 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.

Paul and Prakash (2005) conducted two experiments to assess the timing, synchrony of ovulation and pregnancy rate in Murrha buffaloes (*Bubalus bubalis*) treated with the Ovsynch (GnRH- $PGF_2\alpha$ - GnRH) protocol. In first experiment, ten non lactating cycling buffaloes were treated with 10 µg of GnRH analogue i.m. (Buserelin acetate) without regard to the stage of estrus cycle, followed by 25 mg of $PGF_2\alpha$ i.m. (Dinoprost tromethamine) 7 days later while second treatment of same GnRH analogue (10 µg i.m.) was given 48 h after $PGF_2\alpha$ 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±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 per cent for TAI and 30.70 per cent for buffaloes inseminated following spontaneous estrus.

Bhosrekar (2006) synchronized four hundred graded Murrha buffalo suffered from post partum anoestrus (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, $PGF_2\alpha$ on day 7 and second dose of GnRH on the day 9, whereas A. I. was carried out 14 h 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 per cent and 37.5 per cent 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 $PGF_2\alpha$ (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 per cent) were conceived.

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

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+ $PGF_2\alpha$), Group-III (Ovsynch 33) and Group-IV (Ovsynch 48). Buffaloes from Group-I were inseminated where as buffaloes from remaining groups were injected with 10 ug of GnRH analogue (Buserlin acetate) and 25 mg of $PGF_2\alpha$ (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 $PGF_2\alpha$ 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 $PGF_2\alpha$ was 55.3± 2.11, 55.3 ± 2.37 and 51.8 ± 2.49 h in Groups-II, III and IV, respectively while percentage of induced estrus was 80, 100 and 100 per cent in Group-II, III and IV, respectively. The first service conception rate was 30, 50, 30, and 40 per cent, respectively in Group-I, II, III and IV, respectively.

Roy and Prakash (2008) treated eleven buffaloes with Ovsynch protocol i.e. administrating 10 ug of GnRH analogue (Buserlin acetate) at any stage of estrus cycle (d 0) followed by 25 mg $PGF_2\alpha$ (Dinoprost tromethamine) on (d 7) and second GnRH treatment 48 h after $PGF_2\alpha$ administration and fixed time A.I. at 12 h and 24 h post second GnRH administration. Following Ovsynch treatment, 82 per cent (9/11) buffaloes exhibited estrus.

Warrich *et al*. (2008) studied the timing of ovulation and pregnancy rate in Niliravi buffaloes in $PGF_2\alpha$ induced luteolysis and Ovsynch protocol. The mean time of ovulation after the onset of standing estrus was greater (30.6 ± 1.5 h) in $PGF_2\alpha$ 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 per cent in low breeding season, respectively.

Ghuman *et al.* (2009) treated eleven true anoestrus buffalo heifers in summer season with Ovsynch protocol. Ovsynch protocol involved administration of GnRH analogue at days 0 and 9, and $PGF_2\alpha$ 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 per cent ($p < 0.05$) 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 $PGF_2\alpha$ (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), $PGF_2\alpha$ (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 per cent for Group-I and 63.6; 57.1 per cent for Group-III whereas 0 percent for control non-treated group, respectively.

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.

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 (Fertigyl) to the buffaloes irrespective of the stage of estrus cycle (d 0), followed by 20 ug of $PGF_2\alpha$ (Lutalyse) (d 7), and second GnRH treatment 10 ug 48 h after $PGF_2\alpha$ (d 9). Estrus was detected twice daily using two vasectomised bulls. The number of buffaloes exhibited estrus were 91.7 (43/47) per cent.

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 $PGF_2\alpha$ (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 per cent 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 pluriparious buffalo cows (11 cyclic and 10 acyclic) were selected and administered 100 ug GnRH i/m at day 0, 500 ug $PGF_2\alpha$ i/m at day 7, and100 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 per cent (2/12) and 0 per cent (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 per cent.

Oropeza *et al*. (2010) selected eighty multiparous lactating Murrha 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) on d 0 followed by 25 mg of $PGF_2\alpha$ (Dinoprost methamine) on d 7 and second injection of 10 ug GnRH on d 9. Buffaloes in the presynch-Ovsynch protocol received two $PGF_2\alpha$ 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 per cent (30/40) and 84 per cent (34/40) whereas conception rate was 39 per cent and 53 per cent in Ovsynch and presynch-Ovsynch group, respectively.

Navarange *et al.* (2012) synchronized twelve normal cyclic buffaloes by Ovsynch protocol consisting 2.5 ml GnRH (Receptal) on day 0, 2 ml $PGF_2\alpha$ (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 anoestrus buffaloes using ovsynch and selectsynch 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.PGF_2^{α} @ 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.PGF_2^{α} @ 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 induction 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 induction 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 anoestrus and repeat breeding buffaloes. On this experiment, total fifty multiparous 90-180 days postpartum having average body condition score of which twenty true anoestrus, twenty repeat breeders and ten normal cyclic (control) buffaloes were selected. Twenty anoestrus buffaloes were treated initially once intramuscularly with Sodium acid phosphate 2gm and multivitamin AD_3E injection 10 ml. Ten anoestrus 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 $F_2\alpha$ ($PGF_2\alpha$) 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 buserelin acetate-gonadotropin releasing hormone (GnRH) 20 µg. 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 anoestrus buffaloes. Mid-cycle $PGF_2\alpha$ treatment resulted in 90 per cent estrus induction and 40 per cent conception rate at induced estrus, while buserelin acetate-GnRH 20 µg 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 per cent.

Nakrani *et al.* (2014) studied efficacy of controlled breeding techniques in anoestrus buffaloes. In this experiment, total fifty-five buffaloes more than ninety days postpartum anoestrus 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 multimineral boluses for oral supplementation to their animals @ one bolus daily for seven days. All anoestrus buffaloes were randomly subjected and treated with three standard hormonal protocols (CIDR, Ovsynch and Crestar, n=15 each), keeping a

group of untreated anoestrus control (n=10) and a group of normal cyclic control (n=10). All the fifteen (100 per cent) buffaloes in each group under CIDR, Ovsynch and Crestar protocols exhibited induced oestrus within mean intervals of 65.00±1.55, 69.46±1.04 and 46.00±1.37 hrs, respectively, from PGF2α injection. The conception rates obtained at induced oestrus with CIDR, Ovsynch and Crestar protocols were 46.67, 53.33 and 33.33 per cent, respectively. In untreated anoestrus 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 anoestrus buffaloes using modified Ovsynch based timed insemination protocols. In this experiment, total 50 post-partum anoestrus 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 µg at day 0 followed by cloprostenol 500 ugm on day 7 and second dose of GnRH (10ugm) on day 9. In Group II (Modified Ovsynch protocol I) administered i/m buserelin acetate 20 µg at day 0 followed by cloprostenol 500 ugm on day 7 and second dose of GnRH (10ugm) on day 9. In Group III (Modified Ovsynch protocol II) administered i/m buserelin acetate 10 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 ugm) i/m on day 7 and second dose of GnRH (10ugm) 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 ugm) i/m on day 7 and second dose of GnRH (10ugm) on day 9. In group V administration of insulin on day 0,1 and 2, followed by cloprostenol (500 ugm) i/m on day 7 and GnRH (10 ugm) 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 anoestrus buffaloes. A total forty six postpartum true anoestrus 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 AD_3E 10 ml and oral multi-minerals one bolus daily for 7 days. All anoestrus buffaloes were randomly subjected to standard estrus synchronization protocols (TriU-B, Ovsynch and Heatsynch, n=12 each), keeping a group of untreated anoestrus control (n=10) and a group of normal cyclic control (n=10). In anoestrus buffaloes under TriU-B, Ovsynch and Heatsynch 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 anoestrus 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.

2.3 Estrual Cervical Mucus Properties

2.3.1 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 atypical, 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 per cent) buffalo heifers had a very characteristic (grade 3), 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-oestrus 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 (26.67per 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 per cent).

Sharma *et al.* (2011) examined estrual cervical mucus samples from 61 normal cyclic buffaloes at AI. Typical, atypical and nil pattern of cervical mucus was 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 per cent.

Verma *et al.* (2014) studied arbonisation 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 arbonisation pattern.

2.3.2 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 estrum 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 repeater 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 pleuriparous 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 oestrual 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.

2.3.3 Spin barkeit value

Pattabiraman *et al.* (1967) observed the spin barkeit value of cervical mucus from 58 healthy and regular breeder cows in early, mid and late oestrum and found that average spin barkeit value was 19.0 cm in early and mid while 14.0 cm at late stage of estrus.

Shehata *et al.* (1978) observed the spin barkeit value of cervical mucus of 54 cows and 46 buffaloes during estrus and pooled samples of cervical mucus during luteal phase. Cows and buffaloes showed mean spin barkeit value 29.5±0.443 and 26.0±0.298 cm, respectively during estrus and 4.5 and 4.0 cm during luteal phase.

Bishnoi *et al.* (1982) studied the spin barkeit value of cervical mucus of 26 normal and 26 repeat breeder in estrus. The spin barkeit value of normal cows was 12.0 to 22.0 cm (average 16.46 ± 0.58 cm) and 9.0 to 15.0 cm (average 11.50 ±0.33 cm) in repeater cows.

Rao and Rao (1982) studied the spin barkeit value of estrual mucus of 41 samples obtained from crossbred heifers and reported the spin barkeit value was 4 to 9 cm with a mean of 7.25 cm.

Singh and Kharche (1984) studied spin barkeit value 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 mean spin barkeit value was 6.39 ±

0.35, 4.0±0.59 and 5.76±0.26 inches in intense, intermediate and weak estrus group, respectively.

Adhalikar (1986) studied the spin barkeit 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 mean spin barkeit values were higher in ovulated than in anovulated cows and in ovulated groups the values were lower in fertile heat than in infertile and heat in repeat breeders, respectively.

Modi *et al.* (2011) studied spin barkeit value of cervical mucus of 10 normal and 20 repeat breeder Kankrej cows. The average spin barkeit value of normal and repeat breeder cows were 15.3±0.51cm (12 to 19 cm) and 8.00±0.32 cm (5 to 11.5 cm), respectively.

Sharma *et al.* (2011) examined estrual cervical mucus samples from 61 normal cyclic buffaloes at AI. for spin barkeit. The spin barkeit value of mucus averaged 9.35±0.66 cm. It was significantly higher ($P<0.01$) in conceived than non-conceived buffaloes (12.94±0.81 vs 7.91±0.96 cm).

Verma *et al.* (2014) studied spin barkeit value of cervical mucus of 94 Murrah buffaloes and categorized into three groups namely 0 to 8 cm, 8 to 16 cm and 16 to 24 cm. Spin barkeit value of cervical mucus was 18 (19.14%), 39 (41.48%) and 37 (39.36%) in 0 to 8 cm, 8 to16 cm and 16 to 24 cm., respectively, whereas average value of spin barkeit was 14.16±0.60 cm.

2.4 Hematological Profile

Naidu and Rao (1982) collected blood samples from 25 anoestrus heifers and 10 normal cycling heifers for hemoglobin estimation. The mean level of hemoglobin in anoestrus heifers and in cycling heifers was 8.60 ±0.84 and 10.93 ±1.52 g%, respectively.

Shrivastava and Kharche (1986) studied hemoglobin level in normal cycling and anoestrus Murrah buffaloes in Fertivet treated and control group. In treatment group, 20 buffaloes were treated with Fertivet and no treatment was given to buffaloes of control group. Blood samples were collected on 0 day (prior to treatment) and on the day of heat (post

treatment).The mean hemoglobin level was 7.29 and 8.2 g% on 0 day and on the day of heat, respectively in normal cycling buffaloes. In anoestrus buffaloes, the mean hemoglobin level was 7.54 g% before treatment and 7.78 g% on the day of estrus after treatment with Fertivet.

Kumar and Sharma (1991) studied hemoglobin level in 26 non-descript cows during fertile and non-fertile estrus. The average hemoglobin level in fertile heat and nonfertile heat was 11.71 ± 0.21 and 10.21 ± 0.03 g/dl in cows, respectively and hemoglobin concentration was significantly low in ($P<0.01$) in non-fertile group.

Ramkrishana (1997) studied hemoglobin level in 35 normal cyclical and 35 cross bred anoestrus Jersey cows. The mean hemoglobin level was 9.1 ± 0.80 and 10.43 ± 0.031 g% in anoestrus and normal cycling cross bred cows, respectively.

Nayyar *et al*. (1998) studied hematological parameter in buffalo heifers during prepubertal period as related to age at puberty in buffalo heifers. In Group-I (n=10) healthy heifers that was born during winter, having age at puberty (27.5±3 months) and heifers born during spring season, having age at puberty (35±0.8 months) in Group-II (n=10) were selected. The mean level of hemoglobin in buffalo heifers was 11.4 and 11.56 g/dl in Group I and Group II, respectively whereas mean PCV was 33.85 and 34.90% in Group I and Group II, respectively. There was no difference observed between hemoglobin and PCV of normal and delayed pubertal heifers.

Hedaoo *et al.* (2008) studied hematological parameter of 12 cyclic and 12 anoestrus Surti buffaloes. The mean hemoglobin concentration was 12.96 ±0.37 and 9.98 ±0.30 g% in cyclic and noncyclic (anoestrus) buffaloes, respectively whereas mean PCV was 38.58 ±1.14 and 29.25 ±0.82 % in cyclic and noncyclic (anoestrus) buffaloes, respectively. In differential leucocyte count (DLC), the per cent of neutrophils, lymphocytes, monocytes, eosinophils and basophils were 31.25 ±0.56, 58.58 ± 0.92, 6 ± 0.67, 4.16 ±0.26 and 0.16 ± 0.10, respectively in cyclic buffaloes whereas per cent of neutrophils, lymphocytes, monocytes, eosinophils and basophils were 31.16 ±0.97, 56.83± 0.1, 8.58 ± 0.41, 3.16 ±0.20 and 0.25 ± 0.12 in non-cyclic (anoestrus) buffaloes, respectively.

Kumar Sharad *et al.* (2010) studied hematological parameter of 14 anoestrus and 10 normal cyclic Murrah buffaloes. The blood samples were collected at an interval of 10 days three times (total 42 samples) in anoestrus buffaloes and from normal cyclic buffaloes only at time of A.I. The mean hemoglobin concentration was 13.60±0.51 and 12.20±13.60 g% in cyclic and anoestrus buffaloes, respectively. Normal cyclic animals had significantly ($P<0.05$) higher concentration of hemoglobin than those in anoestrus.

Ali and Shukla (2012) studied hematological changes in post partum anoestrus buffaloes during low breeding season. In this experiment, total 24 buffaloes out of which 6 were normal cyclic and 18 were anoestrus more than 90 days were selected. Those buffaloes were per-rectally explored twice, ten days apart to confirm ovarian activity and genital status. The blood samples were collected on 0 day and 3rd day of second per rectal examination. The mean hemoglobin concentration in normal cyclic and anoestrus buffaloes on 0 day was 12.63 ± 0.49 and 9.81 ± 0.21 g %, respectively while it was 12.73 ± 0.49 and 9.87 ± 0.21 g % on day 3rd per rectal examination in normal cyclic and anoestrus buffaloes. The mean hemoglobin concentration in normal cyclic buffaloes was significantly higher ($P< 0.01$) than anoestrus buffaloes on day 0 and 3rd. In DLC, per cent neutrophils, lymphocytes, monocytes, eosinophils and basophils were 27.50 ± 0.91, 64.83± 2.41, 4.00 ± 0.66, 2.33 ± 0.51 1.33 ± 0.30, respectively in normal cyclic buffaloes whereas per cent of neutrophils, lymphocytes, monocytes, eosinophils and basophils were 25.29 ± 2.35, 68.10 ± 2.88, 2.61 ± 0.44, 2.77 ± 0.73 and 0.55 ± 0.29 in anoestrus buffaloes, respectively on the day 0. The per cent of neutrophils, lymphocytes, monocytes, eosinophils and basophils were 27.77 ± 2.18, 65.61± 2.17, 2.44 ± 0.52, 3.27 ± 0.69 and 0.88 ± 0.22 in anoestrus buffaloes whereas per cent of neutrophils, lymphocytes, monocytes, eosinophils and basophils were 27.50± 1.33, 64.50 ± 1.45, 4.50 ± 0.45, 2.66 ± 0.51 and 0.83 ± 0.28, respectively in normal cyclic buffaloes, buffaloes on 3rd day. The mean of neutrophil, lymphocyte, monocyte, eosinophil and basophil count in anoestrus and normal cyclic buffaloes was non-significant.

Kumar *et al. (*2014) studied hematological profile of 20 postpartum anoestrus and twenty normal cyclic Murrah buffaloes. The mean hemoglobin concentration in postpartum anoestrus and normal cyclic buffaloes was 11.72 ± 0.558 and 10.2 ± 0.789 g%, respectively. The mean of PCV in postpartum anoestrus and normal cyclic buffaloes was 42.142 ± 1.874 and 35.446 ± 3.012 %, respectively. The differences in the hemoglobin and PCV between postpartum anoestrus and normal cyclic buffaloes were nonsignificant. In DLC, per cent of neutrophils, lymphocytes, monocytes, eosinophils and basophil were 42.48 ± 2.478, 50.687± 2.17, 4.3 ± 0.504, 2.313 ± 0.566 and 0.14 ± 0.039, respectively in postpartum anoestrus whereas per cent of neutrophils, lymphocytes, monocytes, eosinophils and basophils were 30.72 ± 3.043, 59.325 ± 2.774, 4.725 ± 0.321, 4.9 ± 1.606 and 0.3 ± 0.089 % in normal cyclic buffaloes, respectively. Significantly higher ($P<0.05$) percentage of neutrophils and lymphocytes were recorded in postpartum anoestrus group buffaloes compared to that of normal cyclic buffaloes which indicated that animals in postpartum anoestrus group were suffering from neutrophilia indicating infection.

Kumar Ashwani *et al. (*2015) studied hematological profile in 24 anoestrus post partum Murrah buffaloes (over 90 days) subjected to different treatment. In Ovsynch protocol group, animals were administered with GnRH 20 ug i/m one the day 0, followed by $PGF_2\alpha$ 500 ug i/m on the 7 and GnRH 20 ug i/m on the day 9. The blood samples were collected on day 0 (day of treatment) and the 11 (day of induced estrus). The mean hemoglobin concentration was lower on the day of treatment (8.68 ± 0.23) with Ovsynch protocol and increased significantly ($P<0.05$) on the day of estrus (9.85 ± 0.32). The mean PCV was lower on the day of treatment (30.16 ± 0.79) in Ovsynch protocol and increased significantly ($P<0.05$) on the day of estrus (32.58 ± 1.05).

Kumar Sunil *et al. (*2015) observed mean hemoglobin level 13.34 ± 0.43 and 11.38 ± 0.56 g %, respectively in estrum and anestrum buffaloes which differ statistically significant at ($p<0.05$). In DLC, per cent of neutrophils, lymphocytes, monocytes, eosinophils and basophils were 45.4 ± 4.03, 48.49 ±4.34, 5.5 ± 1.09, 0.49 ± 0.14 and 0.07 ± 0.027, respectively in estrum whereas per cent of neutrophils, lymphocytes, monocytes, eosinophils

and basophils were, 52.69 ± 2.5, 41.81 ±2.74, 4.76 ± 0.72, 0.70 ± 0.19 and 0.016 ± 0.01 in anestrum buffaloes, respectively.

2.5 Biochemical Profile

Hukeri *et al*. (1979) recorded serum cholesterol level in 30 buffaloes before Fertivet treatment (anoestrus condition) and after Fertivet treatment (exhibiting estrus) and the mean serum cholesterol level was 204.44 mg% and 199.20 mg%, before and after treatment and the difference was statistically non-significant.

Naidu and Rao (1982) collected blood samples from 25 anoestrus heifers and 10 normal cycling heifers for biochemical estimation. The mean blood glucose level of normal cycling heifers (51.11 ±5.89 mg%) was significantly higher ($p<0.01$) than that of anoestrus heifers (45.67±3.50 mg%). The mean total serum protein in cycling heifers (9.20 ±1.85 g%) was significantly higher ($p<0.01$) than that of anoestrus heifers (7.17±1.57 gm%).

Derashri *et al.* (1984) studied blood glucose level in 60 Surti buffaloes at estrus, genital infection, anoestrus and pregnancy. The mean blood glucose level was 56.31 ± 6.23 mg% in estrus animals, 59.95 ± 9.05 mg% in buffaloes having genital infection, 62.58±7.65 mg% in pregnant animals and 43.21±6.74 mg% in anoestrus animals. The levels of blood glucose were significantly higher in estrus animals than anoestrus animals.

Agarwal *et al.* (1985) studied blood glucose level in 10 normal cyclic, 7 anoestrus, 13 repeat breeder, 7 normal calving and 8 retained placenta animals. The mean blood glucose level was 84.54 ± 13.37, 62.90 ± 10.55, 78.08 ± 7.56, 66.75 ± 8.15 and 76.57 ± 4.21 mg% in normal cyclic, anoestrus, repeat breeder, retained placenta animals and normal calving animals, respectively. The blood glucose level was significantly lower in anoestrus cows as compared to normal cyclic and repeat breeder cross bred cows. The mean serum total protein was 8.43±1.22, 8.62±1.52, 9.39±0.63, 8.53±0.72 and 9.81±0.50 g% in normal cyclic, anoestrus, repeat breeder, retained placenta and normal calving animals, respectively.

Shrivastava and Kharche (1986) studied blood glucose and serum cholesterol in normal cycling and anoestrus Murrah buffaloes. The

selected animals were divided into treatment and control group. In treatment group, 20 buffaloes were treated with Fertivet, Fertivet with copper sulphate, prajana, Lugol' iodine with utero-ovarian massage. No treatment was given to buffaloes of control group. The blood samples were collected on 0 day (prior to treatment) and on the day of heat (post treatment). The mean cholesterol level was 79.75 and 80.16 mg% on 0 day and on the day of heat, respectively in normal cycling buffaloes while 73.02 and 60.57 mg% on 0 day and on the day of heat, respectively in anoestrus buffaloes. The mean blood glucose level was 59.0 and 52.64 mg% on 0 day and on the day of heat, respectively in normal cycling buffaloes while 54.62 and 69.42 mg% on 0 day and on the day of heat, respectively in anoestrus buffaloes.

Kumar and Sharma (1991) studied serum biochemical constituent in 26 non-descript cows during fertile and non-fertile estrus. The mean total serum protein level in fertile heat and nonfertile heat was 8.45 ± 0.63 and 7.14 ± 0.12 g/dl in cows, respectively. The mean serum cholesterol level in fertile heat was 105.22 ± 2.06 and in nonfertile heat was 99.17 ± 1.39 mg/dl. The mean blood glucose level in fertile heat and nonfertile heat was 59.00 ± 3.39 and 51.11 ± 2.08 mg/dl in cows, respectively.

Purohit and Bishnoi (1993) studied biochemical constituent in 7 post partum anoestrus Rathi cows and 12 anoestrus heifers before (Group-I) and after induction of estrus with Fertivet (Group-II) in anoestrus cows and heifers and 6 Rathi cows which had come into heat without any treatment as control (Group-III). The mean blood glucose level was higher during induced estrus (46.07 ± 5.73 mg %) as well as during normal estrus (47.16 ± 4.66 mg %), when compared with anoestrus group (44.47 ± 3.26 mg %). However, the difference between Group-I and Group-II and that between Group-II and control was non-significant. The average serum total protein values were non-significantly higher during induced estrus (6.25 ± 0.725 g %) when compared with anoestrus (5.99 ± 0.79 g %), however comparison of values of induced estrus and normal estrus (control) showed significantly higher value in control group (7.52 ± 1.29 g %).

Ramkrishana (1997) studied biochemical profile in 35 normal cyclical and 35 cross bred anoestrus Jersey cows. The mean blood glucose levels was 47.09 ± 12.03 and 62.20 ±3.23 mg% in anoestrus and normal cycling cross bred cows whereas mean blood cholesterol levels was

significantly lower (80.94 ± 6.77mg%) in anoestrus as compared to cycling cross bred cows (108.94 ± 4.64mg%). The mean total protein was 5.91 ± 0.398 and 6.85 ± 0.168 g% in anoestrus and normal cycling cross bred cows.

Tandle *et al.* (1997) studied level of serum cholesterol and total protein in 8 non-descript cows which were in estrus and 8 in post partum anoestrus. The mean cholesterol concentration was significantly higher in estrus cows (160±6.78 mg %) than anoestrus cows (94.38±6.59 mg %) whereas, mean serum total protein concentration was significantly higher in estrus cows (7.74±0.37g %) than anoestrus cows (4.41±0.27 g %).

Dantre *et al.* (1998) studied the blood glucose level in normal cycling and delayed pubertal crossbred heifers. The normal cycling cross bred heifers showed higher mean blood glucose level (50.59±2.28 mg%) than delayed pubertal heifers (47.57±2.28 mg%).

Nayyar *et al.* (1998) studied blood biochemical constituents in buffalo heifers during prepubertal period (Group I) and at age of puberty (Group II). The mean level of total protein in buffalo heifers was 6.84 and 7.17 g/dl in Group I and Group II, respectively whereas mean level of cholesterol was 55.05 and 55.095 mg/dl in Group I and Group II, respectively.

Ahmad *et al. (*2004) studied serum biochemical constituent in cyclic, noncyclic and endometric cross bred cows. The mean level of total protein was 9.19 ±0.45, and 15.23 ±0.89 and 19.16±1.00 g/dl in cyclic, noncyclic and endometric cows, respectively. The mean serum glucose level in cyclic, noncyclic and endometric cows were 50.72 ±01.12, 50.56 ±1.13 and 58.08 ±2.59 mg/dl, respectively. The non significant difference was observed in concentration of urea in cyclic (30.88 ±2.42 mg/dl), non-cyclic (33.80 ±3.85 mg/dl) and endometric (37.12 ±3.45 mg/dl) cows. The mean serum cholesterol level was 199.12 ±9.38, 202.96 ±14.84 and 290.72±15.95 mg/dl in cyclic, noncyclic and endometric cows, respectively.

Hedaoo *et al.* (2008) studied biochemical constituents of total 12 cyclic and 12 anoestrus Surti buffaloes. The mean total protein concentration was 7.59 ±0.56 and 8.37 ±0.35 g/dl in cyclic and noncyclic (anoestrus) buffaloes, respectively but difference was non-significant. The mean BUN level was 10.68 ±1.40 and 21.14±1.70 mg/dl in cyclic and noncyclic (anoestrus) buffaloes, respectively.

Bohara and Devkota (2009) studied biochemical profile of 5 cyclic and 8 non-cyclic Murrah cross buffaloes and recorded non significant difference in the level of mean serum cholesterol in cyclic (162.59 ± 20.84 mg/dl) as compared to non-cyclic buffaloes (142.69 ±6.49 mg/dl). The mean serum glucose level was 73.88 ± 7.14 and 66.44 ±7.01 mg/dl in cyclic and non-cyclic buffaloes, respectively. The non significant difference was observed in the level of serum glucose of cyclic and non-cyclic buffaloes. The mean urea concentration in cyclic and non-cyclic buffaloes was 22.13±2.92 and 24.15±1.84 mg/dl, respectively and no significant difference was observed.

Kumar Sharad *et al. (*2010) studied metabolic profile of anoestrus and normal cyclic Murrah buffaloes. The anoestrus buffaloes (n=14) having smooth and inactive ovaries were selected and the blood samples were collected at an interval of 10 days three times (total 42 samples) and from 10 normal cyclic buffaloes only at time of A.I. The mean blood glucose concentration was 63.33 ±11.04 and 54.17±3.88 mg/dl in cyclic and anoestrus buffaloes, respectively. The mean concentration of total protein was 8.76 ±0.48 and 6.32±0.22 g/dl in cyclic and anoestrus buffaloes, respectively. Normal cyclic animals had significantly higher ($p<0.01$) concentration of serum protein as compared to anoestrus animals.

Virmani *et al.* (2011) studied blood biochemical profile with treatment of Ovsynch protocol in 5 post partum anoestrus Sahiwal cows. The blood samples were collected on day 0 , at time of A.I. and the 21 day after A.I. The mean level of total protein was 6.98 ±1.11 and 8.31±0.37 and 7.75±0.48 g/dl in anoestrus, on day of estrus and on day 21, respectively in cows. The mean level of cholesterol was 181.38±16.01, 141.24±23.01 and 149.86±26.01 mg/dl in anoestrus, on day of estrus and on day 21, respectively. The mean level of urea was 31.40 ±2.13, 29.20±1.87 and 27.20±1.56 mg/dl in anoestrus, on 0 day, day of estrus and on day 21, in post partum anoestrus Sahiwal cows, respectively.

Ali and Shukla (2012) studied biochemical changes in post partum anoestrus buffaloes during low breeding season. In this experiment, total 24 buffaloes out of which six were normal cyclic and 18 were anoestrus more than 90 days were selected. These buffaloes were per-rectally explored twice, ten days apart to confirm ovarian activity and genital status. The blood

samples were collected on 0 day and 3rd day of second per rectal examination. The mean level of serum cholesterol in normal cyclic and anoestrus buffaloes was 130.86±3.80, 74.83±2.06 mg/dl on day 0 and 132.49±3.39, 75.32±1.92 mg/dl on 3rd day, respectively. The mean level of serum glucose in normal cyclic and anoestrus buffaloes was 71.85±2.44, 50.54±1.86 mg/dl on day 0 and 72.38±2.65, 51.39±2.24 mg/dl on 3rd day, respectively.

Jayachandran *et al.* (2013) studied blood biochemical status in cyclic and post partum anoestrus buffaloes. The buffaloes which had not expressed estrus signs for more than five months were taken as true anoestrus (n=43) while ten regular cycling buffaloes were selected as control. There was non significant variation in mean plasma glucose level between anoestrus (51.86±1.01 mg/dl) and regular cyclic buffaloes (56.90±3.30 mg/dl). The mean total cholesterol level in anoestrus and regular cyclic buffaloes was 136.39±4.19 and 167.07±6.03 mg/dl, respectively whereas buffaloes in anoestrus state had significantly ($p<0.01$) lower total cholesterol level.

Kumar Ashwani *et al. (*2015) studied biochemical profile in 24 anoestrus post partum Murrah buffaloes (over 90 days) which were subjected to different hormonal protocols. In Ovsynch protocol group, animals were administered with GnRH 20 ug i/m one the day 0, followed by $PGF_2\alpha$ 500 ug i/m on the 7 and GnRH 20 ug i/m on the day 9. The blood samples were collected on day 0 (day of treatment) and the 11 (day of induced estrus). The mean level of total proteins were increased significantly ($P<0.05$) in anoestrus buffaloes on the day of estrus (6.42 ± 0.29 g/dl) than on the day of treatment (6.03 ± 0.30g/dl) in Ovsynch protocol. The mean cholesterol level was non-significantly ($P>0.05$) higher in anoestrus buffaloes on the day of estrus (90.54 ± 4.80 mg/dl) as compared to the day of treatment (84.37 ± 5.72 mg/dl) in Ovsynch protocol.

Kumar Sunil *et al. (*2015) studied biochemical profile in post partum water buffaloes. In this experiment, the blood samples were collected from three different groups of animals i.e. in Group-I (n=10) buffaloes within 40 to 60 days after parturition at estrus, Group II (n=10) postpartum more than 90 days at anoestrum and post partum cystic buffaloes in Group III (n=10). The mean glucose value was 65.27± 5.07, 65.7±5.36 and 71.2 ± 5.05 mg/dl in estrum, anoestrum and cystic buffaloes, respectively whereas the

mean total protein value was 8.21± 0.37, 6.96±0.22 and 7.17 ± 0.23 g/dl in estrum, anoestrum and cystic buffaloes, respectively. The mean cholesterol level in estrum was 108.16± 7.43, 138.51±13.85 and 105.84±13.05 mg/dl in anoestum and cystic buffaloes, respectively whereas, mean urea level was 39.30± 4.2, and 47.05±4.82 and 29.93±.5.5 mg/dl during estrum, anoestum and cystic buffaloes, respectively.

CHAPTER III

MATERIAL AND METHODS

The present research work entitled “Fertility evaluation after Clomiphene citrate and Ovsynch protocol treatment in buffalo heifers” was carried at Pailpada and Katepurna, village of Akola district as well as Purnathadi Buffalo Unit, Akola and Department of Animal Reproduction, Gynaecology and Obstetrics, Post Graduate Institute of Veterinary and Animal Sciences, Akola, Maharashtra. The work was undertaken from February, 2016 to June, 2016.

3.1 Climatic Condition

Akola is located in subtropical region at 22.24 latitude and 77.62 longitudes at an altitude of 307.415 meters above mean level of sea, having extreme climatic condition with minimum temperature in winter season 12 degree Celsius and maximum temperature in summer 47 degree Celsius.

3.2 Source of Buffalo Heifers

A total eighteen buffalo heifers were selected from Pailpada and Katepurna, village of Akola District and Purnathadi Buffalo Unit, Post Graduate Institute of Veterinary and Animal Sciences, Akola, Maharashtra.

3.3 Selection of Buffalo Heifers

Total eighteen sexually mature buffalo heifers in between age of 3.5 to 4.5 years, who attained optimum body weight with normally developed genitelia and exhibited estrus cyclicity were selected.

3.4 Health and Managemental Practices

The selected buffalo heifers were maintained under village condition with similar management condition and vaccinated against Black Quarter, Foot and Mouth disease and Hemorrhagic septicemia. Locally available forages, greens, concentrates, mineral mixture and kadbi-kutti of jawar were fed to buffalo heifers.

3.5 Experimental Procedure

All the selected buffalo heifers were dewormed using injection Ivermectin @ 1 ml per 50 kg body weight subcutaneously and initial treatment with injection vitamin AD_3E&H, 5 ml I/M (5 days apart), injection Toldimphos sodium 20% w/v per ml @ 5 ml I/M (5 days apart), one microelement bolus daily orally for 10 days and chelated mineral mixture 50 gm daily orally. After initial treatment, these buffalo heifers were randomly divided into three groups comprising six buffalo heifers in each group.

Group-I (Clomiphene citrate); n=6

After ten days of initial treatment, two tablets of 1% copper sulphate solution was administered with drinking water to each buffalo heifer. After 30 minutes, 300 mg tablet of clomiphene citrate was administered orally for five consecutive days.

Group-II (Ovsynch protocol); n=6

After ten days of initial treatment, buffalo heifers from this group were treated with Inj. Buserline acetate 10µg i/m on day 0, Inj. Cloprostenol sodium 500 µg on day 7 and Inj. Buserline acetate 10µg i/m on day 9.

Group-III (Control); n=6

The six buffalo heifers from this group were kept as untreated control animals.

3.6 Estrus Detection and Time Required for Exhibition of Estrus

The buffalo heifers from all the three groups were observed for estrus exhibition and the estrus was exhibited by visual examination by observing different estrus symptoms like frequent micturation, bellowing, congestion of vaginal mucus membrane, vaginal discharge, tummification of vulva and raising of tail in morning and evening. The time required for onset of estrus were recorded after last dose of clomiphene citrate (day 5) in Group-I whereas after $PGF_2\alpha$ injection in Group-II.

3.7 Estrual Cervical Mucus Properties

The estrual cervical mucus was collected from buffalo heifers who has exhibited estrus and collected in petri dish before the insemination. The collected estrual cervical mucus was used to study the following properties.

3.7.1 Fern pattern

Two to three drops of well mixed 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.

3.7.2 Hydrogen ion concentration (pH)

The hydrogen ion concentration of cervical mucus was determined by Himedia 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).

3.7.3 Spin barkeit value

Two to three drops of collected cervical mucus was taken on a grease free glass slide and another grease free glass slide was placed over it. The mucus was stretched between two slides by moving second slide away from first one, until mucus breaks. The distance between two slides just

before the breakage of mucus, string was measured through a scale (cm scale).

3.8 Hematological and Biochemical Analysis

For hematological analysis, blood was collected aseptically from jugular vein of each animal with 18 gauge needle on the day of initial treatment, on the day of start of treatment (10^{th} day) and on day of estrus. The blood samples were collected into a screw caped sterilized anticoagulant vial containing EDTA @ 2mg/ml of blood. Hemoglobin was estimated by using Sahli's haemometer method and expressed in gm/dl while packed cell volume (PCV) was observed by using microhaematocrit scale and expressed in percent. A drop of fresh blood sample was taken on grease free slide and spread immediately by the corner of other slide and air dried. The slides were stained with Leishman's stain and observed for differential leucocyte count (DLC) as per standard procedure. For biochemical analysis, 10 ml of blood was collected in sterilized glass tube. The blood samples allowed to clot at room temperature and subsequently transfered to the laboratory. The serum was separated by centrifugation of samples for 3000 rpm for 10 minutes. The serum glucose was estimated immediately, after that samples were stored at -20 ^{0}C. The serum glucose was estimated by using GOD-POD end point assay and kinetic assay. The serum glucose, cholesterol, total protein and blood urea nitrogen (BUN) were estimated on semi-autoanalyser (Autochem 2011) using Span Diagnostic kits. The serum cholesterol was estimated by using CHOD-PAD enzymatic end point assay whereas serum total protein was estimated by Biuret and end point assay. The blood urea nitrogen (BUN) was estimated by NED-dye, initial rate assay.

3.9 Artificial Insemination (A.I.) and Pregnancy Diagnosis

Those buffalo heifers who were responded to the treatment were inseminated with French mini straw as per A.M. and P.M. rule. The inseminated buffalo heifers were diagnosed for pregnancy detection by per-rectal examination after 60 days. First service conception rate is calculated by using following formula.

$$\text{Conception rate} = \frac{\text{No. of buffalo heifers conceived to first service}}{\text{No. of buffalo heifers inseminated}} \times 100$$

3.10 Details of drugs used

Following drugs were used during complete experimental procedure.

3.10.1 Deworming and initial treatment

3.10. 1.1 Injection Ivermectin

Trade name	:	Hitek
Composition (Each ml)	:	(Ivermectin IP 10mg, Benzyl Alcohol IP 15mg and Propylene Glycol IP qs.)
Dose and route of administration	:	@ 1 ml per 50 kg body weight s/c
Presentation	:	Hitek is available in pack of 10 ml
Manufactured by	:	Manufactured by Virbac Animal Health Private Limited, Anderi (East), Mumbai.

3.10.1.2 Injection Vitamin AD_3E & H

Trade name	:	Intavita-H
Composition (Each ml)	:	(Vitamin A 2,50000 IU, Vitamin D3 25,000 IU, Vitamin E 100 IU and 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
Manufactured by	:	Intas Pharmaceuticals Ltd. Matoda. District-Amdabad.

3.10.1.3 Injection Toldimphos sodium

Trade name	:	Tonophosphon
Composition (Each ml)	:	(Toldimphos sodium 20 %, Sodium salt of 4 dimethyl amino 2-methyl phenyl phosphoric acid, 0.2 g/ml)
Dose and route of administration	:	Cattle and buffalo 5 ml, intramuscular
Presentation	:	Tonophosphon is available in pack of 30 ml vial
Manufactured by	:	Intervet India, Private Limited, India.

3.10.1.4 Microelement Bolus

Trade name	:	Totavit bolus
Composition	:	(Each bolus contains – Copper 1.4 gm, Iron 1.4 gm, Manganese 0.56 gm, Zinc 0.56 gm, Iodine 0.14 gm, Cobalt 0.112 gm, Vitamin E 0.05 gm, Selenium 0.008 gm and live yeast q.s.)
Dose and route of administration	:	Cattle and buffalo 1 bolus daily for 10 days
Presentation	:	Totavit is available in pack of 10 bolus
Manufactured by	:	Vet Mankind, Division of Mankind Pharma. Ltd., Okhla Industrial Estate, New Delhi.

3.10.1.5 Chelated mineral mixture

Trade name	:	Totavit strong
Composition	:	(Each 1kg has nutritional value of Vitamin A 750000 IU, VitaminD_3 75000 IU, Vitamin E, 300mg, Niacinamide 1200 gm, VitaminB_6 20mg, Copper 4200 mg, Cobalt 150 mg, Magnesium 6500 gm, Iron 1750 gm, Zinc 9600 gm, Iodine 350 mg, Manganese 1500 gm, Sulphur 9200 gm potassium 150 mg, Sodium 20 mg, Calcium 250 mg, phosphorus 127.5 gm,

		D. L. Methionine 1929 gm, L. lysin 4.40 gm, lactobacillus sporogenes 75 billion CFU, Saccharomyces Cerevisiae 15 billion CFU.).
Dose and route of administration	:	Cattle and buffalo 50 gm orally
Presentation	:	Totavit strong is available in pack of 1 kg
Manufactured by	:	Vet Mankind, Division of Mankind Pharma. Ltd., Okhla Industrial Estate, New Delhi.

3.10.1.6 Injection Buserelin Acetate (GnRH Analogue)

Trade name	:	Injection Ovulanta
Composition	:	(Each ml contains Buserelin Acetate equivalent to Buserelin ph Eur. 4 mcg, benzyl Alcohol 10 mg preservative)
Dose and route of administration	:	Cattle and buffalo 10 mcg intramuscular
Presentation	:	Ovulanta is available in pack of 5 ml
Manufactured by	:	Vet Mankind, Division of Mankind Pharma Ltd., Okhla Industrial Estate, New Delhi.

3.10.1.7 Injection Cloprostenol Sodium ($PGF_2\alpha$ Anologue)

Trade name	:	Repregna
Composition	:	(Each ml contains Cloprostenol Sodium equivalent to Cloprostenol 250 mcg
Dose and route of administration	:	Cattle and buffalo 500 mcg intramuscular
Presentation	:	Repregna is available in pack of 2 ml
Manufactured by	:	Vet Mankind, Division of Mankind Pharma. Ltd., Okhla Industrial Estate, New Delhi.

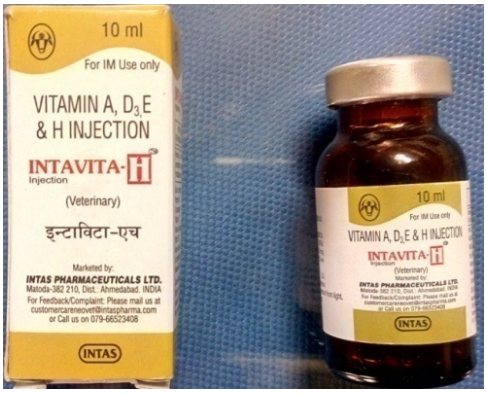
3.10.1.8 Clomiphene citrate

Trade name	:	Ovulanta Kit
Composition	:	(Each uncoated tablet contains clomiphene citrate 300mg Two uncoated tablet contains copper suphate pentahydrate750mg)
Dose and route of administration	:	Cattle and buffalo 300 mg orally for five days after administration of two tablets of copper sulphate
Presentation	:	Ovulanta kit is available in pack of 5 tablets of clomiphene citrate and 10 tablets of $CuSo_{4.}$
Manufactured by	:	Vet Mankind, Division of Mankind Pharma. Ltd., Okhla Industrial Estate, New Delhi.

3.11 Statistical Analysis

Statistical analysis was carried out by using Complete Randomized Design (CRD) and unpaired 't' test by using statistically Web Based Agricultural Statistics Software Package (WASP 1.0).

Injection Hitek



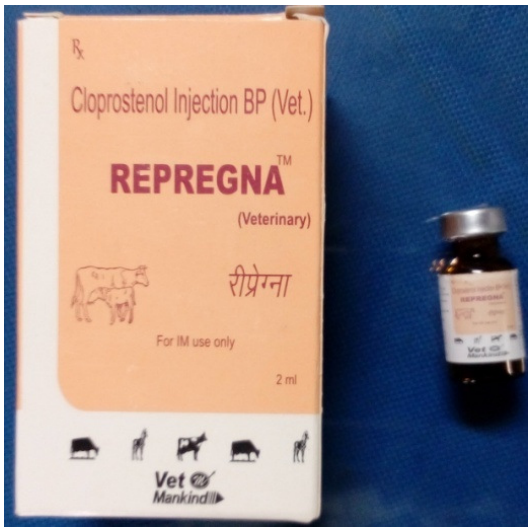
Injection Intavita-H

Chelated mineral mixture

Microelememt bolus

Injection Tonophosphon

Plate 1. Medicines used for initial treatment in present research work



Injection Repregna

Injection Ovulanta

Ovulanta Kit

Plate 2. Drugs and hormones used for treatment in present research work

Plate 3. Initial treatment to buffalo heifer

Plate 4. Biochemical estimation during present research work

Plate 5. Artificial Insemination (A.I.) in buffalo heifer

CHAPTER IV

RESULTS AND DISCUSSION

The present study entitled “Fertility evaluation after Clomiphene citrate and Ovsynch protocol treatment in buffalo heifers” was carried out on eighteen sexually mature buffalo heifers keeping in view with the experimental design described in “Materials and Methods” (Chapter III). The results obtained in the present study are presented in tabular form and discussed under following objectives.

1) Comparitive efficacy of Clomiphene citrate and Ovsynch protocol for estrus induction in buffalo heifers.
2) The time required for exhibition of estrus in buffalo heifers.
3) The estrual cervical mucus properties in buffalo heifers.
4) The hematological and biochemical parameters in buffalo heifers.
5) The first service conception rate in buffalo heifers.

4.1 Comparative Efficacy of Clomiphene citrate and Ovsynch protocol for Estrus Induction in Buffalo heifers

In the present research experiment, total eighteen sexually mature buffalo heifers with optimum age and body weight were randomly selected and divided into three groups comprising six buffalo heifers in each group. Ten days before all the selected eighteen buffaloes were given initial treatment. The selected buffalo heifers (n=6) from Group-I were treated with clomiphene citrate 300mg orally for five consecutive days. Group-II buffalo heifers were treated with Ovsynch synchronization protocol while buffalo heifers from Group-III were kept untreated as control.

In Group-I out of six, three buffalo heifers were exhibited estrus whereas in Group-II out of six, all six buffalo heifers exhibited estrus. In Group-III (Control) none of buffalo heifers has exhibited estrus during experimental period. The percentage of estrus exhibition was 50, 100 and 0 per cent in Group-I, Group-II and Group-III, respectively (Table 1 and Fig.1).

Ovsynch treatment gives better response than clomiphene citrate for estrus induction in buffalo heifers.

Table 1. Comparative efficacy of Clomiphene citrate and Ovsynch protocol for estrus induction in buffalo heifers

Groups (n=6)	Treatments/ protocol	No. of buffalo heifers treated (n=6)	No. of buffalo heifers exhibited estrus	No. of buffalo heifers responded (%)
Group-I (Clomiphene citrate)	Day-0 Initial treatment Day-10, 1 % $CuSo_4$ + Tab. Clomiphene citrate 300 mg for 5 consecutive days	6	3	50
Group-II (Ovsynch protocol)	Day- 0 Initial treatment Day- 10 GnRH Day- 17 $PGF_2\alpha$ Day- 19 GnRH	6	6	100
Group-III (Control)	Day-0 Initial treatment only	6	0	0

The result of present study for estrus exhibition after clomiphene citrate treatment is in concurrence with Deshpande *et al.* (1976), Kankal *et al.* (2008) and More (2012), reported 50 per cent estrus induction rate in anoestrus cows and cross bred cows, respectively. However, the slightly higher estrus induction rate/ response was reported by Purohit and Bishnoi (1993) as 57.1% as compared to present findings in anoestrus cows.

A much higher estrus induction response was reported by Reddy (1999), Hukeri *et al.* (1979) and Deshpande *et al.* (1976) as 85, 85.72 and 100 per cent, respectively in anoestrus buffaloes. Kaikani (1977) and Varma and Kharche (1983) recorded 60.00 percent induction rate in anoestrus cows. Deen and Tanwar (1988) and Kadu and Chede (1992) recorded 66.66 per cent and 68.42 per cent estrus induction response in anoestrus buffaloes while Kurien and Madhavan (1985) recorded 68.42 per cent and 63.64 per cent induction rate in anoestrus cows and heifers.

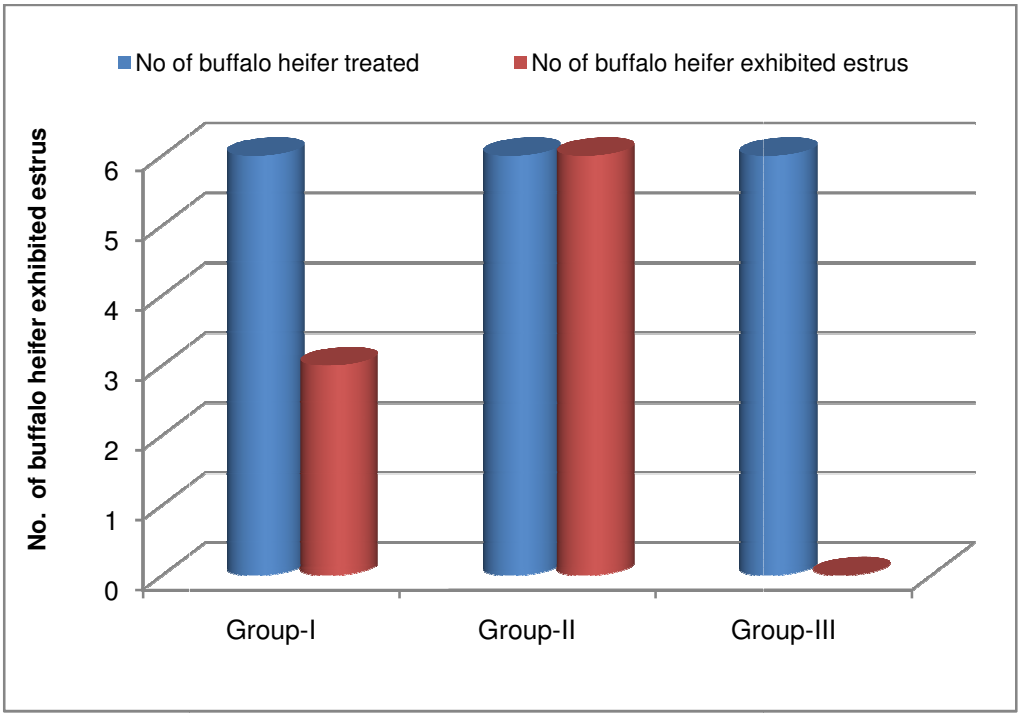


Fig. 1. Estrus exhibition in different treatment groups

The variation in the result of clomiphene citrate for induction of estrus might be difference in the body condition of animals, heat detection methods, season and follicular status of animal at the time of initiation of treatment. The present research experiment is conducted during summer season which might affect the growth of follicles and in turn estrogen production leading to weak or sub-estrus condition in buffalo heifers. Also the gonads are comparatively small compared to pleuriparous animals leading to lesser size follicle and lower estrogen production leading to lower response.

The results of present study for estrus exhibition or synchronization after Ovsynch treatment (Group-II) are in agreement with results obtained by Vijayrajan *et al*. (2007), Ghuman *et al*. (2009), Sharma *et al.* (2010), Navarange *et al.* (2011) and Nakrani *et al*. (2014), reported 100 per cent estrus exhibition in in Ovsynch treated buffaloes. The slightly lower estrus response to Ovsynch treatment than present study were reported by Qin *et al*. (2009) as 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 anoestrus 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 (2008) 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 Murahha buffaloes. Hamman *et al.* (2009) reported 70 per cent in buffalo heifers and Ali *et al.* (2010) reported 50 per cent in post partum anoestrus buffaloes.

The reproductive seasonality of buffalo due to the melatonin secretion as an endocrinology variation is observed during the year (Zicarelli, 1999). The low estrus response as well as ovulation observed to some authors may be due to implementation of the protocol in non breeding season or during summer season. 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 protocol.

In control group, none of the buffalo heifer has exhibited estrus till day 60 from the start of experiment.

4.2 The Time required for Onset of Estrus in Different Treatment Groups

In the present study, the average time required for onset of estrus in buffalo heifers is 136 ± 8.00 hrs in Group-I while 67.83 ± 4.12 hrs in Group-II (Table 2 and Fig. 2). The results are statistical significant at 1% level of significance.

On comparison of results for the present parameter obtained through treatment of clomiphene citrate and Ovsynch protocol, it is found that mean time interval for onset of estrus in Group-II (Ovsynch protocol) is statistical significant than group-I (clomiphene citrate) animals. However, the difference in time interval can be attributed to the method of calculating time interval, as in Group-I, time interval is recorded after administration of last bolus (5^{th} day) and in Group-II time interval can be calculated after administration of $PGF_{2\alpha}$ injection. Also both the treatment are different mode of action.

Table 2. The time interval for onset of estrus in different treatment groups

Groups (n=6)	Treatments/ protocol	No. of buffalo heifers treated (n=6)	No. of buffalo heifers exhibited estrus	Average time required for onset of estrus (hrs)
Group-I (Clomiphene citrate)	Day-0 Initial treatment Day-10 1 % $CuSo_4$ + Tab. Clomiphene citrate 300 mg for 5 consecutive days	6	3	136±8.00**
Group-II (Ovsynch protocol)	Day- 0 Initial treatment Day- 10 GnRH Day- 17 $PGF_2\alpha$ Day- 19 GnRH	6	6	67.83 ± 4.12

** Significant at (P<0.01)

The present findings pertaining to the time required for onset of estrus with clomiphene citrate are in agreement with Dugwekar *et al*. (1980) and Banerjee and Roychoudhary (1989) recorded mean interval of 5 days for inducing estrus in anoestrus cows and Murrah grade buffaloes, respectively with Fertivet. Purohit and Bishnoi (1993) and Reddy (1990) recorded mean interval of 8.75 days and 8.42 ± 098 days in anoestrus cows treated with Fertivet and Clofert Vet, respectively for induction of estrus which is slightly higher than result of present study.

A higher mean time interval of 11.13 days for onset of estrus was recorded by Hukeri *et al*. (1979) in anoestrus buffaloes treated with Fertivet. However, a much higher mean interval of 21 to 25.33 days was recorded by Verma and Kharche (1983) and Reddy *et al*. (1994) for onset of estrus in anoestrus buffaloes treated with Fertivet. From the above observations reported by different research workers, it appears that variation in time interval exists from 5 days to 25.33 days in anoestrus cows and buffaloes for estrus induction. The variation in time interval from the time required for estrus induction after clomiphene citrate treatment might be due to stage of follicular development at the time of start of treatment. As the treatment during late diestrus of second follicular wave may induce estrus earlier comparative to other periods of estrus cycle.

The mean time required for onset of estrus in Ovsynch treated buffaloes (Group-II) observed in present study is 67.83 ± 4.12 hrs. which is in correlation with reports recorded by Nakrani *et al.* (2014) recorded 69.46 ± 1.04 hrs and Neglia *et al.* (2003) who recorded 60 h after $PGF_2\alpha$ injection whereas 70.60 ± 1.30 hr. reported by Buhecha *et al.* (2016) and 70.62 ± 14.82 hr by Giripunje *et al.* (2010) in field buffaloes. The time required for onset of estrus in Ovsynch protocol was cited by Vijayrajan *et al.* (2007) reported 55.3 ± 2.11 hr. Navarange (2011) observed 48.70 ± 0.21 hr while Thorat *et al.* (2012) observed 41.25 ± 2.26 hr in Ovsynch treated Marathwadi anoestrus buffaloes which are slightly less than result of present study. However Ali *et al.* (2010) reported 18.67 ± 3.18 hr for onset of estrus which is lower than the result obtained by the present study. The wide variation noticed the duration of onset of estrus could be due to difference in the developmental stage of the preovulatory follicle at the time of $PGF_2\alpha$ injection. This could also be due to the longer interval from estradiol peak to the onset

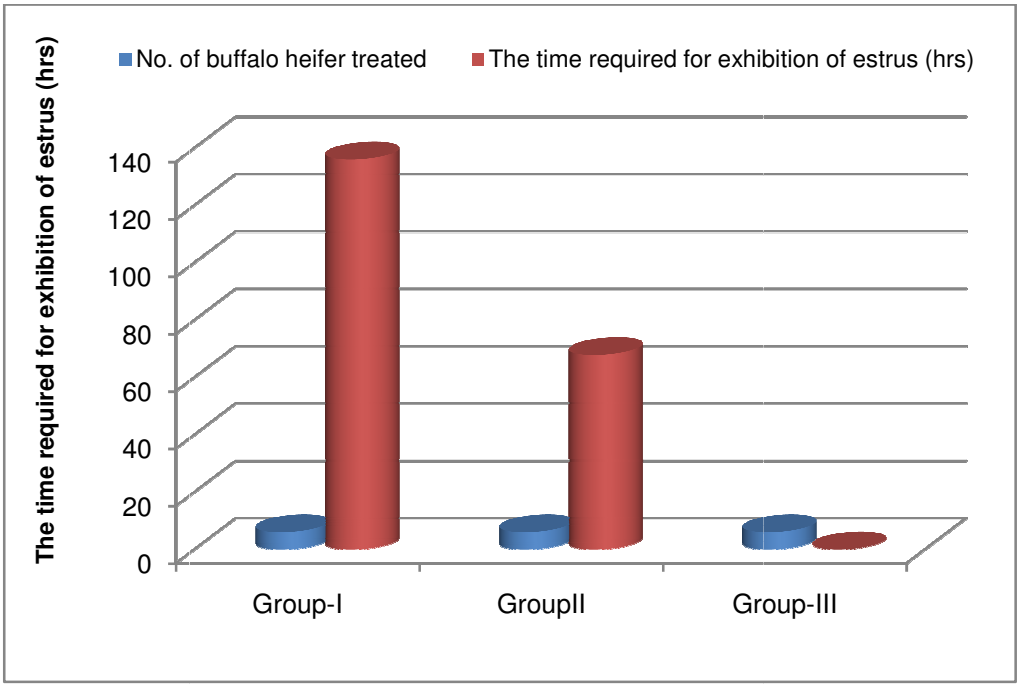


Fig. 2. The time required for exhibition of estrus in different treatment groups

of estrus or to the lower estradiol concentration (Vijayrajan *et al*. 2007). The earlier onset of estrus in Ovsynch protocol could be due to the presence of matured follicle at the time of $PGF_2\alpha$ administration resulting in synchronies onset of estrus.

On comparison of results obtained through the treatment of Clomiphene citrate and Ovsynch protocol, it was found that mean time interval for the onset of estrus in Group-II (Ovsynch protocol) animals was lower than Group-I buffalo heifers which is statistically significant at ($p<0.01$) level.

4.3 Estrual Cervical Mucus properties

Cervical mucus discharge was collected during estrus before A.I. from Group-I and Group-II buffalo heifers and the properties like fern pattern, hydrogen ion concentration (pH) and spin barkeit was evaluated.

4.3.1 Fern pattern

In the present study, cervical mucus samples were collected from Group-I and Group-II buffalo heifers and typical, atypical and nil type of fern pattern was studied. 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 (Table 3).

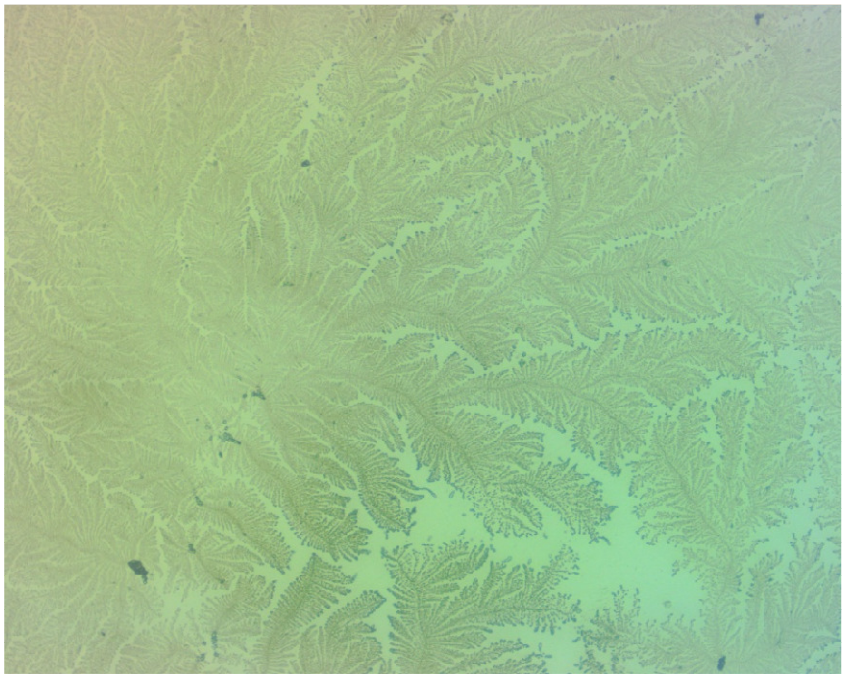
Table 3. Fern pattern of cervical mucus of buffalo heifers in different treatment groups

Groups	Fern pattern type (%)		
	Typical	Atypical	Nil
Group-I (n=3) (Clomiphene citrate)	2/3 (66.66)	1/3 (33.33)	0/3 (00.00)
Group-II (n=6) (Ovsynch protocol)	3/6 (50.00)	2/6 (33.33)	1/6 (16.16)
Overall	5/9 (55.55)	3/9 (33.33)	1/9 (11.11)

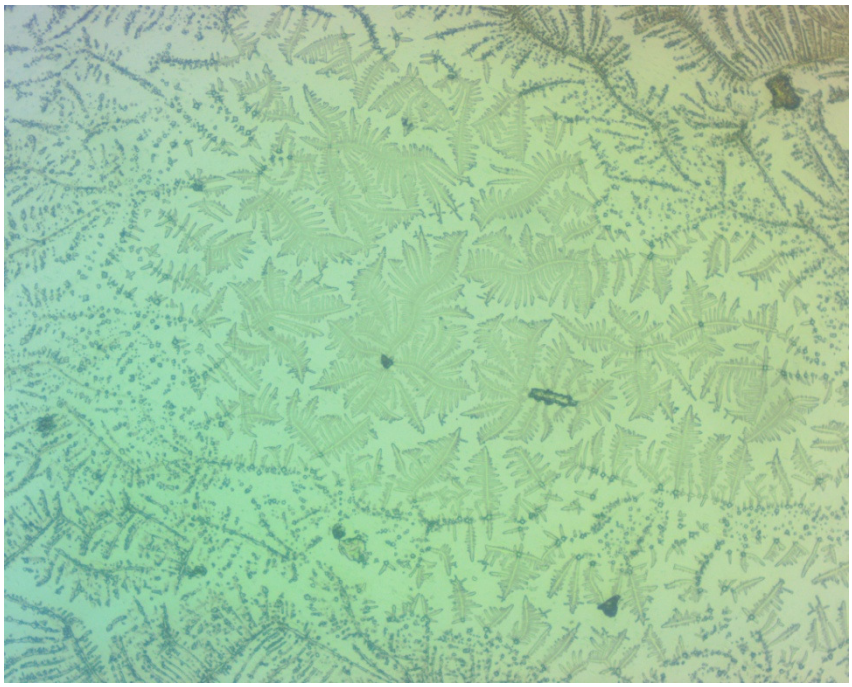
The present findings are slight in agreement with Shrivastav *et al.* (2000) observed typical (63.33 per cent), atypical (26.67 per cent) and nil (10 per cent) type of fern pattern in cows. Varma *et al.* (2014) observed typical (54.25 per cent), atypical (37.24 per cent) and nil (8.51 percent) type of arbonization/ fern pattern in Murrah buffaloes. The present findings are comparable in cows reported by Salphale *et al.* (1993) observed typical (55.56 per cent) and atypical (27.28 per cent) type of fern pattern in estrus synchronized cows. Sharma *et al.* (1987) observed typical (60.46 per cent), atypical (27.91 per cent) and nil (11.93 per cent) type of fern pattern in repeat breeding crossbred cows which is lower than result of present study.

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 and nil patterns 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).

In the present study, higher conception rate (50 per cent) was observed in typical fern pattern in Group-I while 66.66 percent in Group-II which is in agreement with Sharma *et al.* (2011) and Varma *et al.* (2014).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. Also in the present study, higher conception rate was observed in buffalo heifers which may facilitate movement of spermatozoa as compared to nil or dotted type pattern (Alena *et al*. 2008). 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.



Typical fern pattern



Atypical fern pattern

Plate 6. Type of fern pattern of cervical mucus of buffalo heifers

4.3.2 Hydrogen ion concentration (pH)

In the present study, mean pH of cervical mucus is found to be 7.36 ± 0.12 in group-I (Clomiphene citrate) and 7.53±0.15 in group-II (Ovsynch Protocol). The result is statistically non significant at ($p<0.01$) level (Table 4).

Table 4. pH and Spin Barkeit Value of cervical mucus of buffalo heifers in different treatment groups

Sr. No.	Group	Average pH	Average Spin Barkeit Value (cm)
1.	Group-I (Clomiphene citrate) (n=3)	7.36±0.12	9.33±0.66
2.	Group-II (Ovsynch protocol) (n=6)	7.53±0.15	9.5±0.17

The result of present study for pH is in correlation with Pattabiraman *et al.* (1967) recorded the pH of cervical mucus from healthy and regular breeder cows fall within range of 7.0 – 9.0 with the mean of 8.03± 1.22. It tended always to be alkaline and rarely acidic.

Rao and Rao (1982) observed the mean pH of cervical mucus was 7.93 and appeared to be closely associated with fern pattern which is slightly higher. 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.

Slightly lower pH than the present study was reported by Wani *et al.* (1979), Pandy *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.

4.3.3 Spin Barkeit Value

In the present study, Spin Barkeit Value of cervical mucus is 9.33 ± 0.66 cm in group-I buffalo heifers treated with clomiphene citrate and 9.5 ± 0.17 cm in group-II buffalo heifers treated with Ovsynch protocol. The results are statistically non-significant at ($p<0.01$) % level between the groups (Table 4).

The present findings are closely related with the findings reported by of Sharma *et al.* (2011) reported the spin barkeit value of mucus averaged 9.35 ±0.66 cm in normal cyclic buffaloes.

However, much higher spin barkeit value was reported by Bishnoi *et al.* (1982) observed the spin barkeit of cervical mucus of normal cows was 12.0 to 22.0 cm (average 16.46 ± 0.58 cm) as well as Verma *et al.* (2014) observed 14.16 ±0.60 cm in Murrah buffaloes. Pattabiraman *et al.* (1967) observed the spin barkeit value of cervical mucus as 19 cm in early and mid while 14 cm in late stage of estrum.

A much lower spin barkeit value was reported by Rao and Rao (1982) reported 4 to 9 cm spin barkeit value in crossbred heifers with a mean of 7.25 cm. Singh and Kharche (1984) reported spin barkeit value in intense estrus group was highest (6.39 ± 0.35 inches) followed by weak estrus group (5.76±0.26 inches) and intermediate group (4.0±0.59 inches) and reported the difference was highly significant ($p<0.01$).

The variation in the spin barkeit value may be due to stage of estrus, spontaneous induced estrus, species, level of estrogen as well as activity of secretary cells of genital system.

4.4 Hematological Profile

Hematological profile like Hemoglobin, PCV and DLC were studied on day of selection (0 day), after initial treatment (10^{th} day) and on day of estrus from buffalo heifers of all the three groups (Table 5).

Table 5. The hematological parameters in buffalo heifers

Parameters		0 Day (n=18)	10^{th} day (n=18)	Day of estrus (n=9)
Hemoglobin (gm/dl)		8.40 ± 0.36^{a}	9.25 ± 0.45^{ab}	10.42 ± 0.66^{b}
PCV (%)		32.72 ± 1.69	33.16 ± 1.77	37.77 ±2.27
DLC	Neutrophils (%)	27.83 ± 1.30	27.72 ± 1.08	29.66 ± 1.25
	Lymphocytes(%)	62.33 ± 1.56	62.38 ± 1.24	62.77 ± 2.03
	Eosinophils(%)	6.94 ± 1.21^{a}	4.83 ± 0.63^{ab}	3.33 ± 0.23^{b}
	Monocytes(%)	2.16 ± 1.21	3.11 ± 0.52	2.44 ± 0.70
	Basophils(%)	1.00 ± 0.24	1.11 ± 0.24	0.44 ± 0.24

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

4.4.1 Hemoglobin (Hb)

In the present study, the mean of hemoglobin level is 8.40 ± 0.36, 9.25 ± 0.45 and 10.42 ± 0.66 gm/dl on day of selection (0 day), after initial treatment (10^{th} day) and on day of estrus, respectively. The mean level of hemoglobin is statistically significant on the day 0 and on the day of estrus however, non significant on day of 0 and on the of 10^{th} as well as on the of 10^{th} and on the day of estrus at ($p<0.05$).

The present findings are in agreement with Kumar Ashwani *et al. (*2015) recorded Hb concentration was lower on the day of treatment (8.68 ± 0.23 gm/dl) in Ovsynch protocol and increased significantly ($p<0.05$) on the day of estrus (9.85 ± 0.32 gm/dl). Kumar Sunil *et al. (*2015) recorded 13.34 ± 0.43 g% hemoglobin level during estrum and 11.38±0.56 g% Hb during anoestrum in buffaloes. Shrivastava and Kharche (1986) reported mean level of Hb 7.29 and 8.28 g% on the day 0 and on the day of heat after clomiphene citrate which is in concurrence with result of present study. Also Ramkrishna (1997), Hedaoo *et al. (*2008), Kumar *et al. (*2010) and Ali and Shukla (2012) observed the higher level of hemoglobin in cyclic animals than the non cyclic i.e. anoestrus.

In the present study, the level of hemoglobin increased from the day 0 to the day of estrus which may be due to effect of initial treatment.

4.4.2 Packed Cell Volume (PCV)

In the present study, the mean of PCV level is 32.72±1.69, 33.16 ± 1.77 and 37.77±2.27 % on day of selection (0 day), after initial treatment (10 th day) and on day of estrus, respectively. The levels of PCV are statistically non-significant at ($p<0.01$) level when compared in between days of treatment.

The finding is in agreement with Nayyar *et al. (*1998) reported 33.85 and 34.90 % PCV in normal and delayed pubertal buffalo heifers. Slightly higher PCV was reported by Hedaoo *et al*. (2008) as 38.58 ±1.14 and 29.25 ±0.82 % in cyclic and noncyclic (anoestrus) buffaloes, respectively. Slightly lower PCV than the result of present study was reported by Kumar Ashwani *et al. (*2015) on the day of treatment i.e. 30.16 ± 0.79 and increased significantly ($P<0.05$) i.e. 32.58 ± 1.05 % on the day of estrus.

4.4.3 Differential Leucocyte Count (DLC)

In the present study, mean value of neutrophils, lymphocytes, eosinophils, monocytes and basophils are 27.83 ± 1.30, 62.33 ± 1.56, 6.94 ± 1.21, 2.16 ± 1.21 and 1.00 ± 0.24 % on day of selection (0 day), 27.72 ± 1.08, 62.38 ± 1.24, 4.83 ± 0.63, 3.11 ± 0.52 and 1.11 ± 0.24 % after initial treatment (10 th day) and 29.66 ± 1.25, 62.77 ± 2.03, 3.33 ± 0.23, 2.44 ± 0.70 and 0.44 ± 0.24 % on day of estrus, respectively. In the present study, the percentage of eosinophil is statistically differing on day 0 and on the day 10th as well as on the day 10th and on the day of estrus at ($p<0.05$) whereas the other DLC parameters are non-significant compared between the days.

The result of present findings are in concurrence with Hedaoo *et al*. (2008) recorded the per cent of neutrophils, lymphocytes, monocytes, eosinophils and basophils were 31.25 ±0.56, 58.58 ± 0.92, 6 ± 0.67, 4.16 ±0.26 and 0.16 ± 0.10 in cyclic buffaloes whereas per cent of neutrophils, lymphocytes, monocytes, eosinophils and basophils were 31.16 ±0.97, 56.83± 0.1, 8.58 ± 0.41, 3.16 ±0.20 and 0.25 ± 0.12 in non-cyclic (anoestrus) buffaloes. Ali and Shukla (2012) recorded per cent neutrophils, lymphocytes,

monocytes, eosinophils and basophils were 27.50 ± 0.91, 64.83± 2.41, 4.00 ± 0.66, 2.33 ± 0.51 1.33 ± 0.30 in normal cyclic buffaloes whereas per cent of neutrophils, lymphocytes, monocytes, eosinophils and basophils were 25.29 ± 2.35, 68.10 ± 2.88, 2.61 ± 0.44, 2.77 ± 0.73 and 0.55 ± 0.29 % in anoestrus buffaloes, respectively. The mean of neutrophil, lymphocyte, monocyte, eosinophil and basophil count in anoestrus and normal cyclic buffaloes was non-significant. Kumar *et al. (*2014) recorded per cent of neutrophils, lymphocytes, monocytes, eosinophils and basophil were 42.48 ± 2.478, 50.687± 2.17, 4.3 ± 0.504, 2.313 ± 0.566 and 0.14 ± 0.039 in postpartum anoestrus whereas per cent of neutrophils, lymphocytes, monocytes, eosinophils and basophils were 30.72 ± 3.043, 59.325 ± 2.774, 4.725 ± 0.321, 4.9 ± 1.606 and 0.3 ± 0.089 % in normal cyclic buffaloes, respectively. The significantly higher ($P<0.05$) percentage of neutrophils and lymphocytes were recorded in postpartum anoestrus buffaloes compared to that of normal cyclic buffaloes which indicated that animals in postpartum anoestrus group were suffering from neutrophilia indicating infection. Kumar Sunil *et al. (*2015) recorded per cent of neutrophils, lymphocytes, monocytes, eosinophils and basophils were 45.4 ± 4.03, 48.49 ±4.34, 5.5 ± 1.09, 0.49 ± 0.14 and 0.07 ± 0.027 in estrum whereas per cent of neutrophils, lymphocytes, monocytes, eosinophils and basophils were, 52.69 ± 2.5, 41.81 ±2.74, 4.76 ± 0.72, 0.70 ± 0.19 and 0.016 ± 0.01 in anoestrum buffaloes, respectively.

In the present study, statistically significant difference is observed in eosinophil on the day 0 and on the day of estrus which might be due to effect of deworming with ivermectin on the day 0 which reduced the parasitic infestation in buffalo heifers.

4.5 Biochemical profiles

The biochemical parameters like serum cholesterol, serum glucose, serum protein and BUN were studied on the day of selection, after initial treatment (10^{th} day) and on the day of estrus. The mean biochemical values are presented in Table 6.

Table 6. The biochemical parameter in buffalo heifers

Biochemical profile	0 Day (n=18)	10th day (n=18)	Day of estrus (n=9)
Cholesterol mg/dl	62.15 ± 3.54	68.55 ± 3.35	65.17 ± 4.59
Glucose mg/dl	41.83 ± 2.49	44.79 ± 2.13	46.1 ± 2.00
Total protein gm/dl	7.29 ± 0.30	7.31 ± 0.22	7.27 ± 0.19
BUN mg/dl	27.28 ± 1.69^{a}	24.72 ± 1.10^{ab}	19.17 ± 2.37^{b}

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

4.5.1 Serum cholesterol level/ concentration

In the present study, mean value of serum cholesterol is 62.15 ± 3.54, 68.55 ± 3.35 and 65.17 ± 4.59 mg/dl on day of selection (0 day), after initial treatment (10th day) and on day of estrus, respectively. The results are statistically non-significant at ($p<0.05$) compared between the days.

The present finding for serum cholesterol is in slightly agreement with Shrivastava and Kharche (1986) reported 73.02 and 60.57 mg/dl on the day of Fertivet treatment and on the day of heat in anoestrus buffaloes. Nayyar *et al. (*1998) observed 55.05 and 55.09 mg/dl in prepubertal and pubertal buffalo heifers which is slightly lower than the result of present study. Bohara and Devkota (2009) recorded 162.59 ± 20.84 in cyclic and 142.69 ±6.49 mg/dl in non-cyclic buffaloes, Ali and Shukla (2012) recorded 130.86±3.80 in normal cyclic and 74.83±2.06 mg/dl in anoestrus, Jayachandran *et al. (*2013) observed 167.07±6.03 in regular cyclic and 136.39±4.19 mg/dl in anoestrus and Kumar Ashwani *et al. (*2015) reported 84.37 ± 5.72 on the day of treatment and 90.54 ± 4.80 mg/dl on the day of estrus in buffaloes which is higher than the result of present study.

The higher cholesterol levels than the result of present study in cows were also reported by Ramkrishna (1997), Tandle *et al.* (1997), Ahmad (2004) and Virmani *et al.* (2011).

Pregnenolone is the immediate precursor of all steroids which is derived from cholesterol (Hafez, 1980). Cholesterol in turn is synthesized from Acetyl-coA. Acetyl-coA is synthesized by oxidative decarboxylation of pyruvate and a few other reactions. Pyruvate in turn is the product of glycolysis. Therefore concentration of blood cholesterol and in turn steroid synthesis are possibly related to energy status of the animals. As cholesterol

being the precursor of steroid hormone indicates the reproductive status of the animal. It varies in concentration during various stages of reproduction such as lactation, estrus, pregnancy and parturition (Nath *et al.*, 2005).

The mean level of cholesterol reported in the present study are within the normal physiological range in buffaloes. The variation in the level of serum cholesterol might be due to variation in feeding, energy status of animal and level of production.

4.5.2 Serum glucose level/ concentration

In the present study, mean value of serum glucose is 41.83 ± 2.49, 44.79 ± 2.13 and 46.1 ± 2.00 mg/dl on day of selection (0 day), after initial treatment (10^{th} day) and on day of estrus, respectively and results are statistically non-significant at ($p<0.05$) level.

The result of present findings of serum glucose is in correlation with Derashri *et al.* (1984), reported 43.21±6.74 mg/dl in anoestrus Surti buffaloes and Dantre *et al.* (1998) observed 47.5±2.28 mg/dl in delayed pubertal buffalo heifers. Slightly higher level of blood glucose was reported by Shrivastava and Kharche (1986) observed 59.0 and 52.64 mg/dl before the Fertivet treatment and 54.62 and 69.42 mg/dl on the day of heat in Murrah buffaloes. Also Kumar Sharad *et al.* (2010) observed 54.17 ± 3.88 mg/dl glucose in anoestrus buffaloes while Ali and Shukla (2012) recorded 50.54±18.86 mg/dl in 71.85±2.44 mg/dl in cyclic buffaloes. Jayachandran *et al.* (2013) and Kumar Sunil *et al.* (2015) observed 51.86±1.01 and 65.7±5.36 mg/dl glucose during anoestrus buffaloes. The higher blood glucose than the result of present findings were also reported in cows by Agarwal *et al.* (1985) 62.90±10.55, Kumar and Sharma (1991) 51.11±2.08 and Ramkrishna (1997) 47.09±12.03 mg/dl in anoestrus condition.

The blood glucose level influences the pituitary functions. Likewise, FSH hormone is a glycoprotein, glucose is essential for biological activity of hormone. Reduced energy intake and negative energy balance are allotted with decreased maximum diameter of dominant follicle and corpus luteum during the last few cycles before the occurrence of anoestrus (Hafez and Hafez 2000). The anoestrus or delayed pubertal condition denotes the negative energy status which affects the follicular development resulting in follicular atresia and anoestrus. The relative hypoglycemia might possibly affect expression of stress symptoms. Mc Clure (1965) observed that

variations in blood glucose were clearly linked to cyclicity and fertility. The loss of ovarian activity in hypoglycemic animals is due to the effect of hypoglycemic state on the released of gonadotropins from hypothalamus. Morrow (1969) reported that energy deficiency delayed the puberty but did not affect estrus activity after puberty unless severe energy restriction occurs.

The mean level of blood glucose is within the normal physiological range in buffaloes. However, the variation in the level of blood glucose might be due to variation in feeding, body condition, level of production and physiological state of animal.

4.5.3 Serum total protein level/ concentration

In the present study, mean value of serum total protein is 7.29 ± 0.30, 7.31 ± 0.22 and 7.27 ± 0.19 gm /dl on day of selection (0 day), after initial treatment (10^{th} day) and on day of estrus, respectively. The mean value of serum total protein in present study found to be non-significant at ($p<0.05$) level.

The present findings for total protein is concurrence with Nayyar *et al. (*1998) observed 7.17 gm/dl total protein at the pubertal age in buffalo heifers and Hedaoo *et al.* (2008) observed 7.59 ±0.56 gm/dl in cyclic buffaloes. Slightly lower levels of total protein in buffaloes were reported by Kumar Sharad *et al.* (2010) observed 6.32±0.22 gm/dl, Kumar Ashwani *et al.* (2015) 6.42±0.29 gm/dl on the day of Ovsynch treatment and Kumar Sunil *et al.* (2015) observed 6.96±0.22 gm/dl in anoestrus condition. Slightly higher level of total protein than the result of present finding is reported by Hedaoo *et al.* (2008) as 8.37±0.35 gm/dl in anoestrus, 8.76±0.46 gm/dl in cyclic condition and Kumar Sunil *et al.* (2015) observed 8.21±0.3 gm/dl during estrus period.

Agrawal *et al.* (1985) and Ahmad *et al.* (2004) reported higher level of total protein than result of present findings in cows.

The differences reported in total protein levels by different workers could be due to variation in breeds, environment, level of nutrition and reproductive and health status of animals. Excessive intake of protein in the feed can reduced the fertility and increases the number of services per conception.

4.5.4 Blood Urea Nitrogen (BUN) level/ concentration

In the present study, mean value of serum BUN is 27.28 ± 1.69, 24.72 ± 1.10 and 19.17 ± 2.37 mg/dl on day of selection (0 day), after initial treatment (10 th day) and on day of estrus, respectively. The mean level of BUN is statistically significant on the day of 0 and on the day of estrus however, non significant on the day 0 and 10 and the day 10 and on the day of estrus at ($p<0.05$) level.

The result of present findings for BUN is concurrence with Bohara and Devkota (2009) recorded 22.13±2.92 and 24.15±1.84 mg/dl in cyclic and noncyclic buffaloes and Virmani *et al.* (2011) observed 27.20 ±1.57 mg/dl on day of 21 post A.I. in Sahiwal cows. The higher level of BUN was reported by Kumar Sunil *et al. (*2015) observed 39.30 ± 4.2 mg/dl, 47.05±4.82 mg/dl BUN during estrus and anoestrus condition in buffaloes. The lower level of BUN than the present study was reported by Hedaoo *et al. (*2008) recorded 10.68±1.40 and 21.14±1.70 mg/dl in cyclic and non cyclic buffaloes, respectively.

The mean level of BUN in the present study is within the normal physiological range in buffaloes however, statistically significant on the day 0 and during estrus in buffalo heifers at ($p<0.05$) level. The variation in the level of BUN might be due to variation in feeding, level of protein percentage and stage of lactation of animals.

4.6 The First Service Conception Rate in Different Treatment groups

In the present study, one out of three buffalo heifers inseminated is found pregnant with 33.33 per cent conception rate in Group-I while two out of six buffalo heifers inseminated are found pregnant with 33.33 per cent conception rate in Group-II. The first service conception rate is 33.33 per cent in both treatment groups (Table 7).

Table 7. The first service conception rate in different treatment groups

Groups	No. of buffalo heifers	No. of animals responded and inseminated	No. of buffaloes pregnant and Pregnancy rate in per cent
Group-I (Clomiphene citrate)	6	3	1 (33.33)
Group-II (Ovsynch protocol)	6	6	2 (33.33)

In the present study, the first service conception rate after clomiphene citrate treatment is in agreement with Kurien and Madhawan (1985) recorded 30.30 per cent conception rate in heifers treated with Fertivet. The slightly lower conception rate than the result of present study was reported by Deen and Tanwar (1988) recorded 25 per cent conception rate in buffaloes treated after clomiphene citrate.

Purohit and Bishnoi (1993) reported overall conception rate of 100 per cent (50 per cent at first service and remaining 50 per cent at 3 rd service) in anoestrus cows. The higher conception rate than the result of present study were reported by Hukeri *et al.* (1979) 80 per cent, Varma and Kharche(1983) 100 per cent, Banerjee and Roychoudhary (1989) 75 per cent, Kadu and Chede (1992) 61.53 per cent and Ingawale *et al.* (2011) 75 per cent in buffaloes. Also higher pregnancy rate was observed by Dugwekar *et al.* (1980) 80 per cent, Reddy *et al.* (1990) 55 per cent, Purohit and Bishnoi (1993) 50 per cent and Reddy *et al.* (1994) 87.50 per cent in cows. The variations in pregnancy rate might be due to age, nutritional status, method of heat detection, type of breeding technique used for heat detection, season etc.

The present finding regarding first service conception rate in Ovsynch treated buffaloes (Group II) are in agreement with Ali *et al.* (2010) and Buhecha *et al.* (2016) who reported 33.33 per cent conception rate in Ovsynch group, and Paul and Prakash (2005) reported 33.30 per cent and 30. 70 per cent conception rate in Ovsynch treated Murrha buffaloes for TAI and buffaloes inseminated following spontaneous estrus, respectively. Neglia *et al.* (2003) reported 36 per cent and 28 per cent following synchronization

with Ovsynch protocol in cyclic and noncyclic Italian buffaloes during nonbreeding season. Non breeding season is considered an important factor for low conception rate. Warrich *et al.* (2008) reported 36.35 per cent and 34.4 per cent conception rate in breeding and low breeding season in Niliravi buffaloes. Oropeza *et al.* (2010) recorded 39 per cent conception rate in Ovsynch while 53 per cent in presynch Ovsynch group lactating Murrah buffaloes in tropics. Nakrani *et al.* (2014) recorded 53.33 per cent conception rate in anoestrus buffaloes. Kumar *et al.* (2010) reported 53.57 per cent first service conception rate in Ovsynch treated buffaloes bred by natural services using fertile bull.

The lower conception rate than the results of present study was reported by Baruselli (2001) reported pregnancy rate as low as 7 per cent with Ovsynch treatment in anoestrus buffaloes and Karen and Darwish (2010) reported 18 per cent conception rate in cyclic buffaloes whereas 0 per cent in acyclic Egyptian buffaloes during summer. Ghuman *et al.* (2009) reported 18 per cent conception rate in true anoestrus buffalo heifers during summer season. Ingawale *et al.* (2007) reported 23.7 per cent first service conception rate in post partum anoestrus buffaloes. The higher conception rate than the results of present study are reported by Ali and Fahmy (2007) recorded 60.00 per cent conception rate in cyclic buffalo cows inseminated at 14 hr after last GnRH injection. Carvalho *et al.* (2007) reported 61.1 per cent conception rate in Mediterrian buffaloes having body score more than 3. Baruselli *et al.* (2000) who observed higher conception rates in buffaloes that presented better body condition. Berber *et al.* (2002) suggested better conception rates when they are previously selected according to BCS in the beginning of Ovsynch program. Thus it is possible to suggest that the success of the synchronization of ovulation for fixed time insemination in buffalo depends on the good selection on the animals for the body condition score in the beginning of treatment.The difference between conception rates of primaparous and multiparous (35.50% vs 51.00%) demonstrated that the parity is a decisive factor in the efficiency of the protocol (Baruselli *et al.* 2003).

On comparison among the two different groups it was observed that buffalo heifers in Group-I (clomiphene citrate) and Group-II (Ovsynch protocol) recorded same conception rate.

CHAPTER V

SUMMARY AND CONCLUSIONS

The present study entitled “Fertility evaluation after clomiphene citrate and Ovsynch protocol treatment in buffalo heifers” was carried at Pailpada and Katepurna, village of Akola district as well as Purnathadi Buffalo Unit, Akola and Department of Animal Reproduction, Gynaecology and Obstetrics, Post Graduate Institute of Veterinary and Animal Sciences, Akola, Maharashtra. The anoestrus is major infertility problem encountered in buffalo heifers and its intensity is grave during non breeding season. The different treatment regimes are available to treat the anoestrus conditions. Out of these, Clomiphene citrate treatment orally is convenient and cheap treatment whereas, Ovsynch is synchronization of ovulation protocol requires hormones and interest of veterinarian with scheduled administration of hormones. Keeping the major problem of summer anoestrus in buffalo heifers maintained at field level, the research was planned to study the comparative efficacy of Clomiphene citrate and Ovsynch protocol for fertility improvement in buffalo heifers during summer season.

Total eighteen sexually mature buffalo heifers in between age of 3.5 to 4.5 years who attained optimum body weight with normally developed genitelia and exhibited estrus cyclicity were selected. The selected buffalo heifers were maintained under village condition with similar management condition and vaccinated against Black Quarter, Foot and Mouth disease and Heamorragic septicemia. Locally available forages, greens, concentrates, mineral mixture and kadbi-kutti of jawar were fed to buffalo heifers. All selected buffalo heifers were given initial treatment with injection Ivermectin @ 1 ml per 50 kg body weight subcutaneously, injection vitamin AD_3E&H 5 ml I/M (5 days apart), injection Toldimphos sodium 20% w/v per ml @ 5 ml I/M(5 days apart), one microelement bolus daily orally for 10 days and chelated mineral mixture 50 gm daily orally. After ten days of initial treatment, the selected buffalo heifers were divided into three groups comprising six buffalo heifers in each group. In Group-I two tablets of 1% copper sulphate solution was administered with drinking water to each buffalo heifer. After 30 minutes 300 mg tablet of clomiphene citrate was administered

orally for five consecutive days. In Group-II buffalo heifers were treated with 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. Group-III the six buffalo heifers from this group were kept as untreated as control animals.

The buffalo heifers from all the three groups were observed for estrus exhibition and the estrus exhibition was judged by visual examination by observing different estrus symptoms like frequent micturation, bellowing, congestion of vaginal mucus membrane, vaginal discharge, tummification of vulva and tail raising in morning and evening. The time required for onset of estrus were recorded after last dose of clomiphene citrate (day 5) in Group-I whereas after $PGF_2\alpha$ injection in Group-II. For hematological and biochemical study, blood samples were collected aseptically from jugular vein of each animal on the day of initial treatment (0 day),on the day of start of treatment (10^{th}) and on day of estrus. The estrual cervical mucus was collected from buffalo heifers who has exhibited estrus and collected in petri dish before the insemination. The collected estrual cervical mucus was used to study the physical properties such as fern pattern, hydrogen ion concentration (pH) and spin barkeit value. The buffalo heifers who were responded to the treatment were inseminated with French mini straw as per A.M. and P.M. rule. The inseminated buffalo heifers were diagnosed for pregnancy detection by per-rectal examination after 60 days.

5.1 Comparative Efficacy of Clomiphene citrate and Ovsynch protocol for Estrus induction in Buffalo heifers

In Group-I out of six, three buffalo heifers were exhibited estrus whereas in Group-II out of six, all six buffalo heifers exhibited estrus. In Group-III (Control) none of buffalo heifers has exhibited estrus during experimental period. The percentage of estrus exhibition was 50, 100 and 0 per cent in Group-I, Group-II and Group-III, respectively and Ovsynch treatment gives better response than clomiphene citrate for estrus induction in buffalo heifers.

5.2 The Time Required for Onset of Estrus in Different Treatment Groups

The average time required for onset of estrus in buffalo heifers is 136 ± 8 hrs in Group-I while 67.83 ± 4.12 hrs in Group-II. The result is statistically significant at ($p<0.01$) level. The mean time interval for onset of estrus in Group-II (Ovsynch protocol) is lower than group-I (Clomiphene citrate) animals due to difference in mode of action of treatment regimes.

5.3 Estrual cervical mucus properties

5.3.1 Fern pattern

In Group-I, typical, atypical and nil pattern was observed in 2 (66.66%), 1 (33.33%) and 0 (0%) cervical mucus of buffalo heifers, respectively. Typical, atypical and nil pattern was observed in 3 (50%), 2 (33.33%) and 1 (16.66%) buffalo heifers, respectively in Group-II. 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.

5.3.2 Hydrogen ion concentration (pH)

The mean pH of cervical mucus is 7.36 ± 0.12 in group-I (clomiphene citrate) and 7.53±0.15 in group-II (Ovsynch Protocol). The result is statistically non-significant at ($p<0.01$) level between the groups.

5.3.3 Spin Barkeit Value

The mean spin barkeit value of cervical mucus is 9.33 ± 0.66 cm in buffalo heifers treated with clomiphene citrate and 9.5 ± 0.17 cm in buffalo heifers treated with Ovsynch protocol. The results are statistically non-significant at ($p<0.01$) level between the groups.

5.4 Hematological profile

5.4.1 Hemoglobin (Hb)

The mean of hemoglobin level is 8.40 ± 0.36, 9.25 ± 0.45 and 10.42 ± 0.66 gm/dl on day of selection (0 day), on the day of treatment (10 th day) and on day of estrus, respectively. The mean level of hemoglobin is

statistically significant on the day 0 and on the day of estrus however, non significant on day of 0 and on the of 10th as well as on the of 10th and on the day of estrus at ($p<0.05$).

5.4.2 Packed Cell Volume (PCV)

The mean of PCV level is 32.72±1.69, 33.16 ± 1.77 and 37.77±2.27 % on day of selection (0 day), on the day of treatment (10 th day) and on day of estrus, respectively. The level of PCV is statistically non-significant at ($p<0.01$) level when compared in between days of treatment.

5.4.3 Differential Leucocyte Count (DLC)

The mean value of neutrophils, lymphocytes, eosinophils, monocytes and basophils are 27.83 ± 1.30, 62.33 ± 1.56, 6.94 ± 1.21, 2.16 ± 1.21 and 1.00 ± 0.24 % on day of selection (0 day), 27.72 ± 1.08, 62.38 ± 1.24, 4.83 ± 0.63, 3.11 ± 0.52 and 1.11 ± 0.24 % on the day of initial treatment (10 th day) and 29.66 ± 1.25, 62.77 ± 2.03, 3.33 ± 0.23, 2.44 ± 0.70 and 0.44 ± 0.24 % on day of estrus, respectively. In the present study, the percentage of eosinophil is statistically differing on day 0 and on the day 10th as well as on the day 10th and on the day of estrus at ($p<0.05$) whereas the other DLC parameters are non-significant compared between the days.

5.5 Biochemical profile

5.5.1 Serum cholesterol level/ concentration

The mean value of serum cholesterol is 62.15 ± 3.54, 68.55 ± 3.35 and 65.17 ± 4.59 mg/dl on day of selection (0 day), on the day of initial treatment (10 th day) and on day of estrus, respectively. The results are statistically non-significant at ($p<0.05$) compared between the days.

5.5.2 Serum glucose level/ concentration

The mean value of serum glucose is 41.83 ± 2.49, 44.79 ± 2.13 and 46.1 ± 2.00.mg/dl on day of selection (0 day), on the day of initial treatment (10th day) and on day of estrus, respectively and results are statistically non-significant at ($p<0.05$) level.

5.5.3 Serum total protein level/ concentration

The mean value of serum total protein is 7.29 ± 0.30, 7.31 ± 0.22 and 7.27 ± 0.19. gm/dl on day of selection (0 day), on the day of initial treatment (10 th day) and on day of estrus, respectively. The mean value of serum total protein is non-significant at ($p<0.05$) between the different days of treatment.

5.5.4 Blood Urea Nitrogen (BUN) level/ concentration

The mean level of BUN is 27.28 ± 1.69, 24.72 ± 1.10 and 19.17 ± 2.37 mg/dl on day of selection (0 day), on the day of initial treatment (10 th day) and on day of estrus, respectively. The mean level of BUN in the present study is within the normal physiological range in buffaloes however, statistically significant on the day 0 and during estrus in buffalo heifers at ($p<0.05$) level.

5.6 The first Service Conception Rate in Different Treatment Groups

In Group-I, one out of three buffalo heifers inseminated is found pregnant with 33.33 per cent conception rate while two out of six buffalo heifers inseminated are found pregnant with 33.33 per cent conception rate in Group-II. The first service conception rate is in both treatment groups is similar however, efficacy of estrus exhibition is higher in Ovsynch treated buffalo heifers.

Conclusions

Following conclusions are drawn from the present study.

1. Ovsynch treatment is more effective for estrus induction than Clomiphene citrate treatment in buffalo heifers during non-breeding season.
2. The more synchrony of estrus observed in Ovsynch treated buffalo heifers which is convenient to bred group of synchronized animals.
3. Typical fern pattern of estrual cervical mucus has significant relationship for predicting higher conception rate.
4. The mean level of hemoglobin is increased on the day of estrus compared to the day of selection (0 day) which might be due to effect of initial treatment.

5. The first service conception rate is similar (33.33 per cent) in both treated groups indicating both treatments are equally effective however, Clomiphene citrate treatment is cost effective and is convenient for administration to animal owners.

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APPENDIX - I

The time required for exhibition of estrus in different treatment groups

Sample 1

Number of Observations	3
Average	136.000
Standard Deviation	13.856
Variance	192.000

Sample 2

Number of Observations	6
Average	67.833
Standard Deviation	10.088
Variance	101.767

Test results

T - Statistic	:	8.536
T - Table (0.05)	:	2.365
T - Table (0.01)	:	3.499

Samples are significantly different at both 5% and 1% level of significance

APPENDIX-II
pH of cervical mucus of buffalo heifers in different treatment groups

Sample 1

Number of Observations	3
Average	7.367
Standard Deviation	0.208
Variance	0.043

Sample 2

Number of Observations	6
Average	7.533
Standard Deviation	0.378
Variance	0.143

Test results

T - Statistic	:	-0.697
T - Table (0.05)	:	2.365
T - Table (0.01)	:	3.499

Samples are not Significantly different

APPENDIX-III

Spin barkeit value of cervical mucus of buffalo heifers in different treatment groups

Sample 1		
Number of Observations		3
Average		9.333
Standard Deviation		1.155
Variance		1.333
Sample 2		
Number of Observations		6
Average		9.500
Standard Deviation		1.761
Variance		3.100

Test results

T - Statistic	:	-0.146
T - Table (0.05)	:	2.365
T - Table (0.01)	:	3.499

Samples are not Significantly different

APPENDIX-IV

ANOVA for level of hemoglobin in buffalo heifers

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	2	24.722	12.361	3.807	0.030
Error	42	136.370	3.247	-	-
Total	44	-	-	-	-

Co-efficient of variation = 19.70

Treatments were found to be significant at 5% level of Significance CD(0.05) = 1.714

Comparison of Treatment Means with Critical Difference (0.05)

Treatment No.	T 3	T 2	T 1
Treatment Average	10.422	9.250	8.406
Critical Difference (CD) Compared	a	ab	b

APPENDIX-V

ANOVA for PCV level in buffalo heifers

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	2	169.978	84.989	1.611	0.212
Error	42	2215.667	52.754	-	-
Total	44	-	-	-	-

Co-efficient of variation = 21.418

Treatments were found to be Non Significant

APPENDIX-VI

ANOVA for neutrophil % in buffalo heifers

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	2	25.800	12.900	0.570	0.570
Error	42	950.111	22.622	-	-
Total	44	-	-	-	-

Co-efficient of variation = 16.893

Treatments were found to be Non Significant

APPENDIX-VII

ANOVA for lymphocyte % in buffalo heifers

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	2	1.278	0.639	0.018	0.983
Error	42	1525.833	36.329	-	-
Total	44	-	-	-	-

Co-efficient of variation = 9.652

Treatments were found to be Non Significant

APPENDIX-VIII

ANOVA for eosinophil % in buffalo heifers

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	2	87.133	43.567	3.225	0.050
Error	42	567.444	13.511	-	-
Total	42	-	-	-	-

Co-efficient of variation = 68.349

Treatments were found Significant at 5% level of Significance CD(0.05)= 3.497

Comparison of Treatment Means with Critical Difference (0.05)

Treatment No.	T 1	T 2	T 3
Treatment Average	6.944	4.833	3.333
Critical Difference (CD) Compared	a	Ab	b

APPENDIX-IX

ANOVA for monocyte % in buffalo heifers

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	2	8.300	4.150	1.022	0.369
Error	42	170.500	4.060	-	-
Total	44	-	-	-	-

Co-efficient of variation = 77.493

Treatments were found to be Non Significant

APPENDIX-X

ANOVA for basophil % in buffalo heifers

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	2	2.800	1.400	1.470	0.242
Error	42	40.000	0.952	-	-
Total	44	-	-	-	-

Co-efficient of variation = 104.561

Treatments were found to be Non Significant

APPENDIX-XI

ANOVA for serum cholesterol level in buffalo heifers

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	2	383.864	191.932	0.920	0.406
Error	42	8762.392	208.628	-	-
Total	44	-	-	-	-

Co-efficient of variation = 22.293

Treatments were found to be Non Significant

APPENDIX-XII

ANOVA for serum glucose level in buffalo heifers

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	2	145.200	72.600	0.848	0.436
Error	42	3597.359	85.651	-	-
Total	44	-	-	-	-

Co-efficient of variation = 21.122

Treatments were found to be Non Significant

APPENDIX-XIII

ANOVA for serum total protein level in buffalo heifers

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	2	0.007	0.004	0.003	0.997
Error	42	45.963	1.094	-	-
Total	44	-	-	-	-

Co-efficient of variation = 14.335

Treatments were found to be Non Significant

APPENDIX-XIV

ANOVA for serum BUN level in buffalo heifers

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	2	394.201	197.100	4.988	0.011
Error	42	1659.581	39.514	-	-
Total	44	-	-	-	-

Co-efficient of variation = 25.516

Treatments were found Significant at 5% level of Significance CD(0.05)= 5.980

Comparison of Treatment Means with Critical Difference (0.05)

Treatment No.	T 1	T 2	T 3
Treatment Average	27.280	24.721	19.177
Critical Difference (CD) Compared	a	ab	b

VITA

The author, **Wankar Megha Shridharrao** was born on 16th August 1972 at Wardha, Dist. Wardha (Maharashtra State). She passed her Secondary School Certificate (S.S.C.) examination with distinction in the year 1988 from Kesarimal Girls Highschool, Wardha. She passed her Higher Secondary School Certificate (H.S.S.C.) examination in year 1990 from Jankidevi Bajaj College of Science, Wardha.

Being interested in the field of Veterinary science, she was admitted to Nagpur Veterinary College, Nagpur and successfully completed B.V.Sc. and A.H. degree course in the year 1995. She joined State Government service in year 1999 as Livestock Development Officer and successfully completed her duty since15 years.

Due to keen interest in fertility improvement in farm and field animals, she was selected by Department of Animal Husbandry for in-service post graduation. She joined Department of Animal Reproduction, Gynaecology and Obstetrics for her Post Graduation at Post Graduate Institute of Veterinary and Animal Sciences, Akola, Maharashtra Animal and Fishery Sciences University (MAFSU), Nagpur in the year 2014.

She believes that she will use her knowledge in a constructive manner for veterinary profession.

THESIS ABSTRACT

a)	**Title of the thesis (in Capital letters)**	**:**	**"FERTILITY EVALUATION AFTER CLOMIPHENE CITRATE AND OVSYNCH PROTOCOL TREATMENT IN BUFFALO HEIFERS"**
b)	**Full name of student**	**:**	**Wankar Megha Shridharrao**
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**	**:**	2016
f)	**Major subject**	**:**	Animal Reproduction, Gynaecology and Obstetrics
g)	**Total number of pages In the thesis**	**:**	66
h)	**Number of words in the abstract**	**:**	668
i)	**Signature of Student**	**:**	
j)	**Signature, Name and address of forwarding authority**	**:**	

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ABSTRACT

The present study entitled "Fertility evaluation after Clomiphene citrate and Ovsynch protocol treatment in buffalo heifers" was carried on total eighteen sexually mature buffalo heifers in between age of 3.5 to 4.5 years who attained optimum body weight with normally developed genitelia and exhibited estrus cyclicity were selected. All selected buffalo heifers were given initial treatment and after ten days of initial treatment, the selected buffalo heifers were divided into three groups comprising six buffalo heifers in each group. In Group-I two tablets of 1% copper sulphate solution was administered with drinking water to each buffalo heifer. After 30 minutes 300

mg tablet of Clomiphene citrate was administered orally for five consecutive days. In Group-II buffalo heifers were treated with 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. Group-III the six buffalo heifers from this group were kept as untreated as control animals.

For hematological and biochemical study, blood samples were collected from each animal on the day of initial treatment (0 day), on the day of start of treatment (10^{th}) and on day of estrus. The physical properties like fern pattern, hydrogen ion concentration (pH) and spin barkeit value of cervical mucus were studied. The buffalo heifers who were responded to the treatment were inseminated and pregnancy detection was done by per-rectal examination after 60 days.

In Group-I out of six, three buffalo heifers were exhibited estrus whereas in Group-II out of six, all six buffalo heifers exhibited estrus. In Group-III (Control) none of buffalo heifers has exhibited estrus during experimental period. The percentage of estrus exhibition was 50, 100 and 0 per cent in Group-I, Group-II and Group-III, respectively and Ovsynch treatment gives better response than Clomiphene citrate for estrus induction in buffalo heifers. The average time required for onset of estrus in buffalo heifers is 136 ± 8 hrs in Group-I while 67.83 ± 4.12 hrs in Group-II. The result is statistically significant at ($P<0.01$) level.

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. The mean pH of cervical mucus is 7.36 ± 0.12 in Group-I (Clomiphene citrate) and 7.53±0.15 in group-II (Ovsynch Protocol). The result is statistically non-significant at ($P<0.01$) level between the groups. The mean spin barkeit value of cervical mucus is 9.33 ± 0.66 cm in buffalo heifers treated with Clomiphene citrate and 9.5 ± 0.17 cm in buffalo heifers treated with Ovsynch protocol. The results are statistically non-significant at ($P<0.01$) level between the groups.

The mean level of hemoglobin is statistically significant on the day 0 and on the day of estrus however, non significant on day of 0 and on the of 10^{th} as well as on the of 10^{th} and on the day of estrus at ($P<0.05$). The level of PCV is statistically non-significant at ($P<0.01$) level when compared in between days of treatment. In DLC, the percentage of eosinophil is statistically differing on day 0 and on the day 10^{th} as well as on the day 10^{th} and on the day of estrus at ($P<0.05$).

Whereas, the other DLC parameters are non-significant compared between the days.

The mean value of serum cholesterol is 62.15 ± 3.54, 68.55 ± 3.35 and 65.17 ± 4.59 mg/dl, mean value of serum glucose is 41.83 ± 2.49, 44.79 ± 2.13 and 46.1 ± 2.00 mg/dl, mean value of serum total protein is 7.29 ± 0.30, 7.31 ± 0.22 and 7.27 ± 0.19 gm/dl and mean level of BUN is 27.28 ± 1.69, 24.72 ± 1.10 and 19.17 ± 2.37 mg/dl on day of selection (0 day), on the day of initial treatment (10^{th} day) and on day of estrus, respectively.

In Group-I, one out of three buffalo heifers inseminated is found pregnant with 33.33 per cent conception rate while two out of six buffalo heifers inseminated are found pregnant with 33.33 per cent conception rate in Group-II.

प्रबंध सारांश

१. प्रबंधाचे शिर्षक	:	**''क्लोमीफीन सायट्रेट व ओव्हिसिंच प्रोटोकॉलचे उपचारानंतर म्हशींच्या पारड्यांमधील प्रजनन क्षमतेचा अभ्यास करणे''**
२. विद्यार्थ्याचे पूर्ण नांव	:	**वनकर मेघा श्रीधरराव**
३. मुख्य मार्गदर्शकाचे नांव व पत्ता	:	**डॉ. एम. व्ही. इंगवले** सहाय्यक प्राध्यापक, पशुप्रजनन, स्त्रीरोग व प्रसुतीशास्त्र विभाग, स्नातकोत्तर पशुवैद्यक व पशुविज्ञान संस्था, अकोला.
४. प्रदान केली जाणारी पदवी	:	एम.व्ही.एस्सी. (पशुप्रजनन, स्त्रीरोग व प्रसुतीशास्त्र)
५. पदवी प्रदान करण्याचे वर्ष	:	२०१६
६. मुख्य विषय	:	पशुप्रजनन, स्त्रीरोग व प्रसुतीशास्त्र
७. प्रबंधामधील एकुण पाने	:	६६
८. प्रबंध सारांशामधील एकुण शब्द	:	५१४
९. विद्यार्थ्याची सही	:	
१०. प्रबंधक कार्यवाहीस्तव पाठविणाऱ्या अधिकाऱ्याची सही, नाव व पत्ता	:	

(एच. एस. बिराडे)
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स्नातकोत्तर पशुवैद्यक व पशुविज्ञान संस्था,
अकोला.

सारांश

सदर संशोधन प्रकल्प ''क्लोमीफीन सायट्रेट व ओव्हिसिंच प्रोटोकॉलचे उपचारानंतर म्हशींच्या पारड्यांमधील प्रजनन क्षमतेचा अभ्यास करणे'' या विषयावर अभ्यास करण्यात आला. सदर संशोधनामध्ये साडेतीन ते साडेचार वर्षे वयोगटातील लैगींक दृष्टया सक्षम असणाऱ्या एकूण अठरा म्हशींच्या पारड्यांची निवड करण्यात आली. निवड करण्यात आलेल्या सर्व पारड्यांना दहा दिवसांनी निवड करण्यात

आलेल्या सर्व पारड्यांना सुरवातीचा उपचार करण्यात आला. सुरुवातीच्या उपचारानंतर, दहा दिवसांनी निवड करण्यात आलेल्या सर्व पारड्यांना, तीन समान गटांत विभाजण्यात आले. गट क्र. १ - क्लोमीफीन सायट्रेट उपचार, गट क्र. २ - ओव्हीसिंच उपचार, गट क्र. ३ नियंत्रीत गट. गट क्र. १ मधील पारड्यांना कॉपर सल्फेट चे १% द्रावण करून पाण्यासोबत पाजण्यात आले व त्यानंतर ३० मिनीटांनी ३०० मि.ग्रॅ. क्लोमीफीन सायट्रेटची एक गोळी याप्रमाणे पांच दिवस पाजण्यात आली. गट क्र. २ मधील पारड्यांना १० मायक्रोग्रॅम बुसरलीन ॲसीटेड, ५०० मायक्रोग्रॅम क्लोप्रोस्टीनॉल सोडीयम ७ व्या दिवशी व परत १० मायक्रोग्रॅम बुसरलीन ॲसीटेड ९ व्या दिवशी याप्रमाणे मासांमध्ये देण्यात आले. गट क्र. ३ मधील पारड्यांना उपचार न करीता नियंत्रीत गटांत ठेवण्यात आले.

रक्तातील घटक व रक्तातील ग्लुकोज, टोटल प्रोटीन व युरीया नायट्रोजनचे प्रमाण तपासणीकरीता सर्व पारड्यांचे रक्ताचे नमुने निवड केलेल्या दिवशी (० दिवशी), सुरुवातीचे उपचारानंतर १० दिवसांनी व माजाचे दिवशी याप्रमाणे घेण्यात आले.

माजावर आलेल्या पारड्यांचा योनीस्त्राव गोळा करून योनीस्त्रावाचे भौतीक गुणधर्म जसे फर्न पॅटर्न, सामु व स्पिन बारकेट व्हॅल्यू यांचा अभ्यास करण्यात आला. माजावर आलेल्या पारड्यांना कृत्रीम रेतनाद्वारे फळविण्यात आले. गट क्र. १ मध्ये ६ पैकी ३ पारड्या व गट क्र. २ मध्ये ६ पैकी ६ पारड्या माजावर आल्या तसेच नियंत्रीत गटातील एकही पारडी माजावर आली नाही.

गट क्र. १ व गट क्र. २ मधील पारड्यांना माजावर येण्याकरीता सरासरी १३६ ± ८ तास व ६७.८३ ± ४.१२ तास एवढा कालावधी लागला. माजावर येण्याकरीता लागणाऱ्या कालावधी मध्ये लक्षणीय वाढ दिसून आली.

पारड्यांच्या योनीस्त्रावाचे फर्न पॅटर्न करीता निरीक्षण केले असता, ढोबळमानाने टिपीकल, अटिपीकट व निल पॅटर्न चे प्रमाण ५ (५५.५५%), ३ (३३.३३%) आणि १ (११.११%) आढळून आले. योनीस्त्रावाचा सरासरी सामु गट क्र. १ मध्ये ७.३६ ± ०.१२ व गट क्र. २ मध्ये ७.५३ ± ०.१५ आढळून आला.

गट क्र. १ व गट क्र. २ मध्ये स्पिन बारकेट व्हॅल्यूचे सरासरी प्रमाण अनुक्रमे ९.३३ ± ०.६६ व ९.५ ± ०.१७ से.मी. एवढे आढळून आले.

रक्तातील हिमोग्लोबीनचे सरासरी प्रमाणात ० दिवशी व माजाचे दिवसांत लक्षणीय वाढ आढळून आली परंतु १० व्या दिवसांत व माजांचे दिवसांत लक्षणीय वाढ आढळून आली नाही.

रक्तातील पी.सी.व्ही. चे प्रमाणात दिवसांच्या दरम्यान लक्षणीय वाढ आढळून आली नाही.

डि.एल.सी. मध्ये फक्त इवोसिनोफीचे प्रमाणांत ० दिवशी व १०व्या तसेच १० दिवशी व माजाचे दिवशी लक्षणीय फरक आढळून आला परंतु इतर घटकांमध्ये विशेष बदल आढळून आला नाही.

रक्तातील कोलेस्ट्रॉलचे प्रमाण सरासरी ६२.१५ ± ३.५४, ६८.५५ ± ३.३५ आणि ६५.१७ ± ४.५९ मि.ग्रॅ/डे.लि. रक्तातील ग्लुकोजचे प्रमाण सरासरी ४१.८३ ± २.४९, ४४.७९ ± २.१३ आणि ४६.१ ± २.०० मि.ग्रॅ/डे.लि., टोटल प्रोटीनचे प्रमाण ७.२९ ± ०.३०, ७.३१ ± ०.२२ आणि ७.२७ ± ०.२९ ग्रॅम/डे.लि. आणि रक्तातील युरीया नायट्रोजनचे प्रमाण सरासरी २७.२८ ± १.६९, २४.७२ ± १.१० आणि १९.१७ ± २.३७ मि.ग्रॅ./डे.सी. याप्रमाणे ० दिवशी, १०व्या दिवशी व माजाचे दिवशी आढळून आले.

गट क्र. १ मध्ये फळविण्यात आलेल्या तीन पारडंयापैकी एक पारडी (३३.३३%) व गट क्र. २ मध्ये फळविण्यांत आलेल्या ६ पारंड्यापैकी २ पारड्या (३३.३३%) गाभण आढळून आल्यात. दोन्ही गटांमधील पारड्यांचे गाभण राहण्याचे प्रमाण सारखेच आढळून आले.