

**TREATMENT OF ANESTROUS GRADED MURRAH
BUFFALOES (*Bubalus bubalis*) USING CERTAIN
HORMONAL COMBINATIONS BY MONITORING
OVARIAN ACTIVITY**

By

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No part of the thesis has been submitted by the student for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of investigations have been duly acknowledged by the author of the thesis.

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DECLARATION

I, hereby declare that the thesis entitled **“TREATMENT OF ANESTROUS GRADED MURRAH BUFFALOES (*Bubalus bubalis*) USING CERTAIN HORMONAL COMBINATIONS BY MONITORING OVARIAN ACTIVITY”** submitted to Sri Venkateswara Veterinary University, Tirupati for the degree of MASTER OF VETERINARY SCIENCE is the result of original research work done by me. I also declare that the materials contained in this thesis have not been published earlier.

Date: 12-12-2014

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ABSTRACT

Treatment of subestrus and true anestrous lactating graded Murrah buffaloes (*Bubalus bubalis*) using estrus and ovulation hormonal protocols, Double PGF_{2α} (Group A), Ovsynch (Group B) and Norgestomet plus PMSG (Group C) and their influence on fertility during low breeding season was studied. Following the treatment, with Double PGF_{2α} (Group A) 8 buffaloes exhibited estrus to the first PGF_{2α} injection between day 0 to 11 of the treatment protocol. In the other two treatments with Ovsynch (Group B) and Norgestomet plus PMSG (Group C) none of the true anestrous buffaloes exhibited estrus between day 0 to 9. The estrus detection rate was 75 (12/16), 75(12/16)

and 100 (16/16) per cent in Double PGF_{2α} - subestrus, Ovsynch and Norgestomet plus PMSG- true anestrus groups. There was no significant difference in mean time to onset of estrus among the three treatments. Most buffaloes in the present study showed intermediate to weak estrus signs in Double PG (Group A) and Ovsynch (Group B) groups on the contrary, the Norgestomet plus PMSG showed intense to intermediate estrus in more (14/16) number of animals. Peak estrus synchrony with highest number of animals exhibited estrus between 48 to 72h with 7/12 in group A, 8/12 in group B and 10/16 in group C. The artificial submission rate was 75 (12/16), 75 (12/16) and 100 (16/16) per cent in Double PGF_{2α} - subestrus, Ovsynch, and norgestomet plus PMSG respectively. Ovulation rate response of 83.50 (10/12), 91.66 (11/12), 87.50(14/16) per cent was observed in Double PG (Group A) and Ovsynch (Group B) and Norgestomet plus PMSG, respectively. The conception rate at induced estrus in the present study for treatment groups involving Double PG in the treatment of subestrus and Ovsynch and Norgestomet plus PMSG in the treatment of true anestrus buffaloes were 58.33 (7/12), 66.67 (8/12) and 62.50 (10/12) per cent, respectively. However, the overall conception rate was 75 (9/12), 75 (9/12) and 81.25 (13/16) per cent. During this treatment period in the control group, only 36.36 (8/22) and 22.7 (5 /22) per cent anestrus buffaloes exhibited estrus and conceived respectively. The results of present study, with inseminations at observed estrus appears as an effective way of managing reproduction in subestrus buffaloes with luteal activity using double injection prostaglandins and true anestrus buffaloes either non cyclic or in the follicular phase of the estrous cycle with Ovsynch and Norgestomet plus PMSG as we could record acceptable ovulation and conception rates. The treatment of true anestrus buffaloes with Norgestomet plus 500 I.U of PMSG appears to have some beneficial effects with

100 per cent estrus response, intense estrus, in an observable manner with 87.5 per cent ovulation rate and higher overall (81.25%) conception rate.

LIST OF SYMBOLS AND ABBREVIATIONS

AI	Artificial insemination
CIDR	Controlled Internal Drug Releasing device
CL	Corpus luteum
d	day
ELISA	Enzyme linked immunosorbent assay
E ₂	Estrogen
FSH	Follicular stimulating hormone
GnRH	Gonadotropin releasing hormone
g	gram
h	hour
im	intra muscular
inj	injection
kg	kilogram
LH	Luteinizing hormone
L	Low
H	High
PMSG	Pregnant Mare Serum Gonadotropin
TRUS	Trans Rectal Ultrasonography
ml	milliliters

mm	millimeters
EIA	Enzymatic Immuno Assay
IU	International Units
PG	Prostaglandin
MHz	Mega Hertz
MTB	Mini Tab
eCG	Equine Chorionic Gonadotropin
IVSM	Intra Vulvo Submucosal
hCG	Human Chorionic Gonadotropin
mg	milligram
ng	nanogram
nmol	nanomole
pg	picogram
PGF _{2α}	Prostaglandin F ₂ alpha
PRID	Progesterone releasing intravaginal device
P ₄	progesterone
μg	microgram
CG	Chorionic Gonadotropin
SE	Standard Error
SF	Small Follicle
MF	Medium Follicle
LF	Large Follicle
PF	Preovulatory Follicle

CHAPTER I

INTRODUCTION

In buffaloes, prolonged anestrus leads to low reproductive efficiency as expressed by long calving interval exceeding 500 days (Metry *et al.*,1994) and reduction in calf crop, mostly due to lowered ovarian activity (EI-Wishy, 2007). Prevalence of anestrus in buffaloes ranges between 20 to 59 per cent (Kunj *et al.*,2002). Recent transrectal ultrasonography studies of anestrus buffaloes revealed display of follicular turnover in a typical wave like pattern with dominant follicle attaining 12.2 mm diameter (Ghuman *et al.*, 2010). Applying estrus synchronization regimens in anestrus buffaloes may provide a potential alternative for enhancing their reproductive efficiency (Singh *et al.*,2009).The bovine dominant follicle of 10 mm diameter was responsive to exogenous gonadotropins (Wiltbank *et al.*,2002).

Buffalo exhibit seasonality in breeding activity and become sexually active in response to a decreasing day length in late summer to early autumn (Zicarelli,1997). As latitude decreases, calving is more concentrated during the breeding season, providing rapid re-establishment of postpartum ovarian activity and conception (Baruselli *et al.*,2001). Therefore, during the nonbreeding season, buffalo often exhibit a high anestrus incidence, which extends the calving to conception interval and consequently reduces reproductive performance . Consequently, hormonal treatments have been designed to control both luteal and follicular functions, providing exciting possibilities for synchronization of follicular growth and ovulation to enable the use of timed

artificial insemination (TAI) during the nonbreeding season (Singh *et al.*, 1988, Neglia *et al.*, 2003 and De Rensis, 2005). Satisfactory pregnancy rates (approximately 40 to 60%) (Paul and Prakash, 2005) have been achieved with the Ovsynch protocol (Day 0, GnRH; Day 7, PGF_{2α}; Day 9, GnRH and TAI 16 hours after the second GnRH injection (Pursley *et al.*, 1995) in cycling buffalo synchronized during the breeding season. However, anestrous buffalo respond poorly to the Ovsynch protocol (Baruselli *et al.*, 2003) and have lower pregnancy rates after TAI during the nonbreeding season (Baruselli *et al.*, 2003)

In previous studies in postpartum anestrous cows, exogenous progesterone (P₄) increased LH pulse frequency during and after the treatment period (Rhodes *et al.*, 2002). In addition, in comparison with untreated animals, there is increased follicular fluid and circulating estradiol concentrations, LH pulsatile release, numbers of LH receptors of granulosa and theca cells in preovulatory follicles (Rhodes *et al.*, 2002)

Many researchers induced estrus in anestrous buffaloes using different hormonal combinations using PGF_{2α}, GnRH and progesterone (Chohan, 1998 and Kumar *et al.*, 2013). Estrus induction treatments used till date has achieved partial success with respect to conception rates in anestrous buffaloes (Ghuman *et al.*, 2012). This justifies the need to establish a dominant follicle size with exogenous hormonal treatment that can consistently generate an acceptable ovulation and conception rate.

Treatment with ear implant P4 devices (Norgestomet) combined with eCG at device removal has been used in buffaloes (Presicce *et al.*, 2005) and increased ovulation rates and pregnancy rates were recorded. Equine chorionic gonadotropin (eCG) treatment has been suggested as an effective tool for increasing pregnancy outcomes in buffaloes with a high incidence of postpartum anestrous (Rao and

Sreemannarayana,1985 and Murugavel *et al.*, 2009). However, there are few reports of ovarian follicle response and luteal function in the subsequent diestrus of lactating buffalo cows treated with eCG during the nonbreeding season.

Since there is paucity of information, the present study is undertaken to monitor the follicular events that take place consequent to different hormonal treatments with the following objectives in graded Murrah buffaloes.

Objectives of investigation

1. To evaluate the ovarian response in anestrus buffaloes by using GnRH and PGF_{2α} combination as Ovsynch (GPG) treatment.
2. To evaluate efficacy of Ovsynch and Crestar plus PMSG treatments on dominant follicle formation and its ovulation.
3. Estimation of steroid hormones during the course of experiment.
4. Comparison of above treatments in terms of ovarian response and conception rates.

CHAPTER II

REVIEW OF LITERATURE

2.1 HISTORICAL BACKGROUND OF ESTRUS SYNCHRONIZATION

The history of estrous cycle synchronization and the use of artificial insemination in cattle is a testament, to how discoveries in basic science can be applied to advance the techniques used for livestock breeding and management (Beal, 2002).

The first successful estrous synchronization in cattle was reported in 1948 (Christian and Casida, 1948). Synchronizing estrous cycles of domestic cattle depends on control of the functional lifespan of corpus luteum (Hansel and Convey, 1983).

There are two ways to facilitate control of the corpus luteum that result subsequently in estrus and ovulation.

The first method involves long term administration of progestin with subsequent regression of the corpus luteum (Britt, 1987). Estrus and ovulation occur within 2 to 8 days after progestin withdrawal. The second method involves the administration of a luteolytic agent that shortens the normal life span of the corpus luteum. This is accompanied generally with estrus and ovulation within 48 to 120 hours after injection.

2.2 PRINCIPLES OF ESTRUS SYNCHRONIZATION

The hormones used to pharmacologically control the estrous cycle identical to (analogs) the reproductive hormones found within the hypothalamus (GnRH), ovary (estradiol and progesterone) and uterus (PGF_{2α}) of buffalo. The biological activity of

exogenous hormones recapitulates the biological activity of endogenous hormones in the normal buffalo.

Chronology of significant developments in the applied animal endocrinology with reference to estrus synchronization (Murugavel, 2009)

Year	Land mark
1923	Allen and Doisy isolated and synthesized estrogens.
1927	Hammond reported that removal of the corpus luteum from the cow's ovary is followed by estrus and ovulation.
1929	Corner and Allen synthesized progesterone.
1948	Christian and Casida reported the successful synchronization of estrus cycle in cattle by using daily injection of progesterone.
1964	Kattenbach <i>et al.</i> , reported luteolytic property of estrogens.
1966	Mauleon and Rey administered progestogen intra vaginally by means of impregnated sponge pessaries.
1966	Dziuk and coworkers used silastic implants containing melengestrol acetate for synchronization of estrus in cows.
1971	Schally <i>et al.</i> , reported that GnRH from hypothalamus regulates the release of LH and FSH.
1972	Several workers reported the luteolytic action of PGF _{2α} in cattle.
1973	Scham <i>et al.</i> , reported that administration of GnRH induces ovulation.
1976	Niswender and others proposed the mechanism of luteolytic action of prostaglandin.
1979	Rao and Rao reported the use of PGF _{2α} 11 days apart to induce estrus in buffaloes.
1986	Saini <i>et al.</i> , reported the use of potent progestogen (Norgestomet) ear implants.

1991 Singh and Madan reported the use of prostaglandin for estrus synchronization and the treatment of anestrus and subestrus buffaloes.

2.3 INCIDENCE OF POSTPARTUM ANESTRUS

After calving the uterus has to involute, ovaries must commence its cyclical activity in order to continue its reproductive process. The ovarian activity was found to be resumed within 22 to 29 days after calving (Usmani *et al.*, 1985a).

Functional disorders of the ovaries constituted 65 per cent and more of the causes of infertility (Rao and Sreemannarayana, 1982; Singal *et al.*, 1984 and Lensch, 1990). Inactive and non functional ovaries is one of the major or most important causes of anoestrus in buffaloes (Samad *et al.*, 1984; Singal *et al.*, 1984; Vale *et al.*, 1994; Newer *et al.*, 1999 and Baruah *et al.*, 2000). A wide range between 8 and 36 per cent was reported in anoestrous buffaloes 45 to more than 240 days postpartum (Chauhan and Singh, 1979; Shah *et al.*, 1990; Banerjee *et al.*, 1992)

Ovarian inactivity is more frequent in buffaloes on low level of feeding than in those giving high level and also in summer than other seasons (Rao and Sreemannarayana, 1982; Chauhan *et al.*, 1984). The postpartum anestrus when extended to six months or more is known as prolonged anestrus or up to one year known as dead anestrus (Roberts, 1971). Many workers have reported very high incidence of anestrus in various breeds of buffaloes in field condition or at organized dairy farms (Luktuke and Sharma, 1978; Banerjee and Roychowdary, 1992 and Janakiraman, 1988).

The per cent incidence of postpartum anestrus in buffaloes recorded by different scientists in different states varied from 8.9 (Shah *et al.*, 1990); 14.9 (Luktuke *et al.*, 1973); 17 (Narinder Singh *et al.*, 1979; Banerjee *et al.*, 1992) and 28.42 (Rao and Sreemannarayana, 1982).

Low concentration of progesterone and estrogen were found up to ten months postpartum in Jaffarabadi buffaloes and the ovaries remained smooth and inactive. Similar results were reported by Narayan *et al.*, (1984), Madan (1988) and Jain and Pandey (1985) in Murrah buffaloes.

In Surti buffaloes Hedao *et al.*, 2008), reported the overall percentage of non cyclic buffaloes was found to be highest in summer season (59.70%) and lowest in spring (Feb - Apr) season (36.75%) and Chauhan *et al.*, (1979) recorded 46.6% respectively. These differences in incidence of anestrus were attributed to variation in the environmental condition, managerial practices and nutritional status.

2.3.1 INCIDENCE OF SUBESTRUS IN BUFFALOES

The incidence of silent heat or subestrus occurred more commonly in Indian cows and to a greater degree in milch buffaloes (Sane *et al.*, 1967) with 6 - 73 per cent incidence reported under small farm conditions (Shah *et al.*, 1990). The number of subestrus buffaloes were highest (32.6 per cent) during the autumn (Chauhan *et al.*, 1984) followed by the winter (28.4 per cent).

2.4 HORMONES USED IN ESTRUS SYNCHRONIZATION

Induction / synchronization of estrous cycle in buffaloes usually based on prostaglandins or its analogues and progestogen. To improve the efficiency of synchronization treatments based on progesterone or prostaglandins follicular growth

and corpus luteum regression are synchronized by administration of estrogen (estradiol valerate, estradiol benzoate and estradiol cypionate), GnRH and its analogues.

2.4.1 PROGESTOGENS

Christian and Casida (1948), using daily injections of progesterone, were first to record the successful synchronization of estrous cycle in cattle, various methods of administration of progestin were tested and generally shown to be effective at synchronizing estrus. In estrus control and more particularly, in the treatment of anestrus in buffaloes, Intravaginal devices impregnated with progesterone (PRIDs and CIDRs) and ear implants impregnated with potent progestogen (norgestomet) have been tested (Saini *et al.*, 1986; Singh *et al.*, 1988; Subramanyam and Devarajan, 1991; Luthra *et al.*, 1994; Ravikumar *et al.*, 2005; Bartolomeu *et al.*, 2007; Caesar *et al.*, 2011; Azawi *et al.*, 2012 and Naseer *et al.*, 2012). In some trials, workers resorted to administering progesterone by daily injection. Chohan *et al.*, (1995) injected anestrus buffaloes daily with 50 mg progesterone for 14 days.

The results reported by workers such as Shah *et al.*, (1987), treated anestrus buffaloes with Synchronate-B (3 mg norgestomet ear implant + injection containing 3 mg norgestomet and 5 mg estradiol valerate) and the results were encouraging. Norgestomet (17 α -acetoxy-11-beta-methyl 19-nor-preg-4-ene 3-io-dione) ear implant was able to restore ovarian activity, resulting in ovulation in the majority of buffaloes with acceptable conception rates (Chaatry *et al.*, 1998; Deka *et al.*, 2010).

Both exogenous and endogenous progesterone suppress estrus and ovulation by inhibiting LH release (Peter and Lamming, 1984) and its withdrawal resulted in a

gradual rise in plasma LH concentration culminating in a preovulatory LH surge (Garcia -Winder *et al.*, 1986).

Short term progesterone treatments for 7 days (Santosh Kumar *et al.*, 2005 and Malik *et al.*, 2010), 9 days (Rao and Sreemannarayana, 1983 and Shah *et al.*, 1987) and 10 days (Markandeya and Bharkad, 2002; Patel *et al.*, 2003; Anand Singh *et al.*, 2004 and Pawshe *et al.*, 2004) resulted in high proportion of anestrus buffaloes exhibited estrus within 3 to 5 days after withdrawal of the ear implant.

2.4.2 ESTROGENS

Studies have demonstrated that estradiol when administered early in the bovine estrous cycle have shown luteolytic activity (Wiltbank, 1966). Further it was reported that administration of estrogens can induce preovulatory LH surge, ovulation (Lammoglia *et al.*, 1998). In recognition of these properties of estradiol and it was incorporated in short term (9 - 12 days) progesterone treatment to produce normal fertility during estrus induction and synchronization.

2.4.3 PROSTAGLANDINS

In buffaloes, the effect of PGF_{2α} administration is very similar to that observed in cattle (Dhaliwal *et al.*, 1987 and Chohan *et al.*, 1995). The administration of PGF_{2α} from about day 5 of its estrous cycle causes regression of corpus luteum. Thereafter, progesterone declines rapidly to basal concentrations within 24 hrs resulting estrus induction and ovulation. Single or double regimens of PGF_{2α} in buffaloes induce estrus and ovulation in about 60 - 80 per cent of animals during the breeding season (Brito *et al.*, 2002). The mean interval from PGF_{2α} treatment to estrus reported was 88h

and treatment to ovulation was 100h (Bachlaus *et al.*, 1980; Dhaliwal *et al.*, 1987; Chohan *et al.*, 1995 and Brito *et al.*, 2002).

Pregnancy rates following PGF_{2α} treatment are 40 - 50 per cent (average 31-33) and appear to be similar to those obtained after natural estrus (Kamonpatana *et al.*, 1979 and Pathiraja *et al.*, 1979). The efficacy of this treatment is drastically reduced during the non breeding season. The time interval between PGF_{2α} treatment and the onset of estrus in buffaloes varies according to the stage of follicular development at the time of PGF_{2α} administration.

Animals treated when follicles are in the predominance stage display estrus 4 - 6 days later, where as animals treated in the presence of a developing follicle display estrus 2 - 3 days after PGF_{2α} administration.

Application of PGF_{2α} in the treatment of subestrus buffaloes was studied (Pant and Singh, 1991) with 77.5 per cent estrus induction and 54.8 per cent conception at induced estrus.

2.4.4 GONADOTROPIN RELEASING HORMONE (GnRH)

GnRH was found to regulate the secretion of luteinizing and follicular stimulating hormones (Schally *et al.*, 1971). The induction of ovulation of ovarian follicles was demonstrated in lactating cows (Britt *et al.*, 1974) following injection of GnRH. GnRH induced effect is indirect (Chenault *et al.*, 1990) through their induced release of LH (Britt *et al.*, 1974) and FSH (Foster *et al.*, 1980) from anterior pituitary. Later GnRH agonists were developed, which were more potent than native GnRH (Thatcher *et al.*, 1993)

Synchronization of follicular wave and selection of new large follicles following GnRH at any stage of estrous cycle was used as a tool to develop estrus synchronization for fixed time AI (Twagiramungu *et al.*, 1995a)

In cattle, GnRH administration is widely used to manipulate pattern of ovarian follicle development. The administration of GnRH leads to an LH surge during any stage of the estrus cycle, which will promote the ovulation of a developing follicle or induce luteinization and / or atresia of predominant follicle (Guilbault *et al.*, 1990).

2.4.5 PREGNANT MARE SERUM GONADOTROPIN (PMSG)

In 1930, Cole and Hart reported a substance found in the serum of pregnant mares collected between 37 and 131 day of gestation that induced pronounced ovarian growth response in immature rats. This substance was called pregnant mare serum gonadotropin (PMSG), and now is appropriately termed eCG because it is of chorionic origin, which is more consistent with other chorionic gonadotropins (CG) produced during pregnancy in primates. It is synthesized from the chorionic girdle cells initially, and later, from the endometrial cups cells (Thway and Wolfe, 2001). Unique to eCG is its ability to elicit both FSH- and LH-like responses in non-equine mammals. Equine CG has a longer half-life than FSH and hCG because of its glycosylation, which has practical application in its prolonged physiological activity (Matsui *et al.*, 1994). The FSH-like properties of eCG and its availability have led to its application as a convenient exogenous hormone used to stimulate follicular growth and superovulation in laboratory and farm animal species (Mc Dougall *et al.*, 1995).

The equine chorionic gonadotropin (eCG) treatment on ovarian follicular response, luteal function and pregnancy in buffaloes subjected to a timed artificial insemination (TAI) protocol during the nonbreeding season Treatment with eCG at

removal of the Intravaginal P₄ device in a TAI protocol increased the maximum diameter of the dominant follicle, ovulation rate, CL growth rate, functionality after TAI and pregnancy outcomes in lactating buffalo. Thus, administration of eCG at P₄ device removal should be considered to enhance the reproductive efficiency in lactating buffalo (*Bubalus bubalis*) subjected to TAI programs during the nonbreeding season (Carvalho *et al.*, 2013).

It has been suggested that anestrus cows treated with the current TAI protocols might ovulate smaller follicles that produce low P₄ concentrations after ovulation, altering the synthesis or release of uterine PGF_{2 α} and compromising pregnancy recognition and maintenance (Macmillan *et al.*, 2003). Buffaloes treated with eCG had increased maximum diameters of ovulatory follicles at the end of the synchronization protocol.

Equine Chorionic Gonadotropin increased ovulation rate in buffalo subjected to TAI (Singh *et al.*, 1983). In buffalo, Murugavel *et al.* (2009) reported a greater ovulation rate in controlled Intravaginal drug release (CIDR) with eCG (81.0%) compared with CIDR (47.4%) treatment in noncyclical buffalo cows. Anestrus cows have insufficient LH pulsatile release to support the final stages of ovarian follicular development and ovulation. The efficiency of eCG is related to its FSH- and LH-like activities (Murphy and Martinuk, 1991) which ensure continuation of ovarian follicular growth in cows with compromised gonadotropin secretion. In this regard, Baruselli *et al.*, confirmed that the effect of eCG was more pronounced with deeper anestrus in *Bos indicus* breeds and improved fertility after eCG supplementation with increased diameter of the ovulatory follicle.

2.5 ESTRUS SYNCHRONIZATION IN ANESTRUS BUFFALOES

Artificial control of the estrous cycle provided an efficient means of increasing the reproductive capacity of buffalo. Progesterone or norgestomet containing devices (injections PRID, Pessaries, CIDR and ear implants) along with PMSG, estradiol and/or PGF_{2α} have been used successfully to improve synchrony of estrus and conception in buffaloes (Rao and Rao, 1979, 1983; Rao *et al.*, 1985; Singh *et al.*, 1983; Saini *et al.*, 1986 and Chohan *et al.*, 1995). Results of single or double regimens of PGF_{2α} in buffalo and comparable to those obtained in cattle (Kamonpatana *et al.*, 1979; Pathiraja *et al.*, 1979; Pant and Singh, 1980; Dhaliwal and Sharma, 1990).

The endocrine changes following PGF_{2α} induced luteolysis appear to be similar to those occurring at natural estrus (Kamonpatana *et al.*, 1979)

Better synchrony and conception rates have been documented after synchronization during breeding season (winter) than the non breeding season (summer) but remarkably high percentage of (88%) subestrus buffaloes expressed standing estrus after PGF_{2α} treatment during hot summer months (Sahasrabudhe and Pundit, 1997).

2.5.1 GnRH - PROSTAGLANDIN TREATMENTS

In India (Punjab), Takkar *et al.*, (1999), demonstrated significantly early resumption of ovarian activity in buffaloes treated with 12μg of GnRH in early postpartum (30 days) period which helped in reducing the incidence of silent heat and early resumption of ovarian activity.

In Pakistan, Rafiq (2001) made an attempt to induce estrus in Nili-Ravi pluriparous buffaloes using exogenous hormonal treatment consisting of intramuscular injection of GnRH (50μg) on day 30 postpartum followed by 150μg of PGF_{2α} seven

days later and recorded 27.8 per cent estrus response with a low conception rate (16.7%) during the low breeding season (December to August).

2.5.2 OVSYNCH (GnRH - PGF_{2α} – GnRH)

The ovsynch protocol, a sequence of GnRH, PGF_{2α} and GnRH treatments (Pursley *et al.*, 1995) as successfully synchronize ovulation in lactating cows resulting in fertility to timed artificial insemination that was similar to the cows inseminated after detection of estrus.

Ovsynch was used during the period of seasonal anestrus in buffaloes (Baruselli, 2001). In cattle synchronization of stage of estrus with ovsynch is associated with pregnancy rates of between 30 - 40 per cent although pregnancy rates of 20 per cent have also been reported (Thomson, 1999).

The efficacy of ovsynch estrus synchronization protocol was tested in Italian mediterranean buffaloe cows by Neglia *et.al.* (2003) during transition to seasonal anestrus (January) and the buffaloes were at 182 ± 82 days postpartum. The conception rate was 36 per cent with FTAI. The responded animals in this protocol had a follicle greater than 1 cm and tonic uterus. Mucus and estrus behavior were only observed on rare occasion.

Efficacy of ovsynch protocol for synchronization of ovulation and fixed time AI in Murrah buffaloes reported by Paul and Prakash (2005). On the basis of milk progesterone and trans-rectal palpation (60 days post artificial insemination) the conception rate was 33.3 per cent. Ovsynch protocol effectively synchronized ovulation in Murrah buffaloes and resulted in conception rates that were comparable to those achieved with a single AI after detection of spontaneous estrus.

In Italy, Presicce *et.al.* (2005) successfully synchronized ovulation in Mediterranean Italian buffaloes using ovsynch protocol with a conception rate of 42.8 per cent.

In Thailand, Chaikun *et.al.* (2010) evaluated fertility in swamp buffaloes after synchronization of ovulation with ovsynch treatment combined with fixed time artificial insemination (FTAI) and observed 100 per cent estrus response and 51.4 per cent conception rate.

In buffaloes the ovsynch protocol has been observed to synchronize ovulation. In Indonesia, Yendraliza *et.al.* (2011) investigated the effect of administration of GnRH and PGF_{2α} on onset of estrus and conception rate in postpartum swamp buffaloes and reported 100 per cent estrus response, onset of estrus of 37.4 h to 38.4h after PGF_{2α} administration, duration of estrus as 16.8 to 18.2h and 100 per cent conception rate (GnRH + PGF_{2α}).

In Bulgaria, Yotov *et.al.* (2012) assessed the therapeutic effect of ovsynch (GPG) in Bulgarian Murrah buffaloes with inactive ovaries during the low breeding season and achieved overall pregnancy rate of 42.2 per cent and concluded that these treatment could reduce days open and calving interval (PRID + PGF_{2α} + PMSG + hCG and Ovsynch).

2.5.3 NORGESTOMET EAR IMPLANT (CRESTAR)

In India, Rao and Sreemannarayana (1982) conducted field tries to induce ovulatory estrus in non - cycling buffaloes during low breeding season using a 9 day Crestar ear implant (containing 6mg norgestomet intra muscular + injection of 5mg of estradiol valvate + 3mg norgestomet at the time of implant insertion and 600 IU PMSG

at the implant removal. In this trial the estrus induction was present and conception rate at induced estrus was 36%. The above study indicated that norgestomet treatment could synchronize estrus in noncyclic cows during the low breeding season with an acceptable conception rate.

A fertility treatment trial during summer months conducted by Shah *et.al.* (1987) on anestrus Nili-Ravi buffaloes using a subcutaneous ear implant containing 3mg of norgestomet (Synchromate-B, Intervet) 9 days treatment in combination with 600 IU PMSG with fixed time artificial insemination at 48 and 72 h after implant removal recorded an ovulation rate of 70 per cent and conception rate of 53.3 per cent. The parity wise conception rate was 40, 44.4 and 72.2 per cent in first, second and third and higher parity respectively. This indicates that norgestomet treatment regimens were able to restore ovarian activity, resulting in ovulation in the majority of anestrus buffaloes (70%).

Numerous researchers have evaluated the potential of using a norgestomet - estradiol treatment has a tool to synchronize estrous cycle in anestrus buffaloes (Singh *et al.*, 1983, Sanchez *et al.*, 1995, Kojima *et al.*, 1992) demonstrated that synthetic progestins have less activity than progesterone for physiological function other than suppression of estrus.

Singh *et.al.* (1988) documented beneficial efficacy of PRID in conjunction with PMSG which ensured good estrus response (88-90%) and conception rate (50-60%) during summer months in graded murrah buffaloes under village conditions.

Yadav *et.al.* (2001) reported the efficacy of short term progestogen, norgestomet and $\text{PGF}_{2\alpha}$ with PMSG, GnRH on induction of estrus and ovulation with higher conception rate of 83.33 per cent (Norgestmet + $\text{PGF}_{2\alpha}$ + GnRH combination) in

acyclic Murrah buffaloes. In this study, it was opined that supplementation of exogenous progesterone before prostaglandin treatment may be beneficial for better response in buffaloes during summer season.

Anand Singh *et.al.* (2004) studied the effect of crestar (norgestmet ear implant) and its combination with folligon on postpartum anestrous buffaloes under field conditions and recorded 87.5 per cent estrus response and estrus duration 2.142 days with Crestar alone and the same were 87.5 per cent and 3.428 days with crestar and Folligon combinations, indicating that both the treatments could be effectively used to induce estrus in postpartum anestrous buffaloes.

The efficacy of crestar (crestar 10 days protocol) in different breeding seasons in cyclic buffaloes was tested by Pawshe *et al.*, (2004) and observed 100 per cent estrus response during monsoon and summer seasons and 88.23 per cent during winter season. The average time for onset of estrus was 38.78 ± 3.36 , 40.40 ± 3.5 and 55.56 ± 2.98 hours during monsoon, winter and summer seasons respectively. The conception rate varied between the average length of synchronized estrus was 23.28 ± 1.44 h during monsoon season, 22.35 ± 1.20 hours during the winter and slightly more 28.33 ± 1.74 hours during summer with no significant difference.

In Iran, Rastegarnia *et.al.* (2004), synchronized the follicular wave of estrus cycles using norgestomet (Crestar, Intervet) ear implant (17α acetoxy - 11β methyl - 19 nor preg - 4 en - 3, 20 dione) for 12 days in combination with $PGF_{2\alpha}$ (Prosolvin) on day 0 and 17 of the norgestomet insertion. On day 6th of the experiment 50 or 100 μ g Gonadorelin (within 48hr) followed by emergence of a new follicular wave (within 72hr) after GnRH injection.

Srivastava (2005), conducted an experiment using PGF_{2α} and progesterone and estrogens in true anestrous Murrah buffaloes (no palpable CL or follicle on ovaries) during summer months (April, May and June). The estrus induction rate and conception rate after PGF_{2α} injection was 50 and 40 per cent respectively. The same after second PGF_{2α} injection was 60 and zero per cent respectively, where as the estrus response and conception rate with P₄ and E₂ treatment were 75 and 50 per cent respectively.

2.5.4 NORGESTOMET EAR IMPLANT (CRESTAR) + PMSG

The efficacy of norgestomet along with PMSG (eCG) in treating the true anestrus buffaloes was evaluated. Rao *et.al.* (1982), Singh *et.al.* (1988), Agarwal *et.al.* (2001), Anand Singh *et.al.* (2004), Nayak *et.al.* (2009), Kumar *et.al.* (2010) Caesar *et.al.* (2011) and Mahesh *et.al.* (2011) reported 100, 100 (87 in May), 71.42, 87.5, 100, 86.4, 100 and 70.00 per cent estrus response respectively.

The conception rate in anestrus buffaloes using this treatment was 36.00, 50.00, 75.00, 33.33, 85.70 and 70.00 per cent respectively.

The time interval to onset of this treatment reported as 3.42, 2.75 days and 65.14±11.39 hours by Anand Singh *et.al.* (2002), Nayak *et.al.* (2009) and Caesar *et.al.* (2011) respectively. Presicce *et.al.* (2005) reported 88.2 per cent ovulation rate during non breeding season.

2.6 EFFECT OF PROGESTERONE LEVEL ON SYNCHRONIZED ESTRUS

Available reports show that higher progesterone concentration at the time of administration of prostaglandins is associated with delayed onset of estrus (Larson and Bell, 1992). It has also been reported that estrus was manifested in higher percentage of cows (84%) that had high progesterone concentration (>3.1 ng/ml) on the day of the last PGF_{2α} injection than the cows (56%) with low progesterone levels.

The level of progesterone prior to ovulation following administration of PGF_{2α} affect at the fertility of cows in synchronized estrus. Cows conceiving to artificial insemination at induced estrus had higher progesterone levels during preceding luteal phase than those do not conceiving (Folman *et al.*, 1990).

2.6.1 EFFECT OF DIFFERENT PGF_{2α} ANALOGUES ON ESTRUS RESPONSE AND FERTILITY

The fertility of estrus induced with different analogues of prostaglandin was reported to be similar to that of estrus induced with natural PGF_{2α} (Seguin *et al.*, 1985). However, EI-Menoufy and Abdou (1989) reported that the estrus synchronization rate was higher in cows treated with cloprostenol (90%) when compared to natural PGF_{2α} (82%).

2.6.2 ROUTE AND DOSE OF PGF_{2α} ADMINISTRATION

PGF_{2α} has been administered usually by intra muscular injection, various other routes viz. intravenous (Stevens *et al.*, 1995), subcutaneous (Brogliatti, 2000) and through ischio-rectal fossa (Colazo *et al.*, 2002).

Several reports are available regarding administration of reduced dose of PGF_{2α} given into various locations in the reproduction tract namely intra vulvo submucosal (IVSM) (Dhande and Kadu, 1994), intraovarian (Bermubaz *et al.*, 1999), deposition into cervix or vulval lips (Galina and Arthur, 1990), intra uterine infusion (Chatterjee *et al.*, 1989) and injection into the uterine wall (Inskeep, 1973).

Though it is widely accepted that the intra muscular dose of PGF_{2α} and its analogues for estrus synchronization is 25mg and 500μg respectively in cattle (Lagor, 1977), several workers attempted reduced dose of PGF_{2α} with normal or below normal estrus response and fertility (Plata *et al.*, 1989 and Rivera *et al.*, 1994).

2.7 DEGREE OF SYNCHRONY

One of the major advantage of in synchronization of estrus in buffaloes is fixed time of insemination. The dynamics of synchrony should be high to achieve an acceptable pregnancy rate compared with that of insemination at detected estrus (Xu and Burton, 1998).

Rao and Sreemannarayana (1983) observed that 98.2 per cent of buffaloes exhibited estrus within 36 to 80 hrs after implant removal in norgestomet protocol.

Jindal *et.al.* (1988) stated that the estrus was induced in 80 per cent of buffaloes between 72 and 96 hrs after PGF_{2α} administration. Similarly Dhaliwal *et.al.* (1988) and Rajeshwaran *et.al.* (1992) observed estrus in 72 and 87.5 per cent buffaloes within 136 -144 hrs after PGF_{2α} injection.

2.8 SERUM HORMONAL PROFILE

2.8.1 SERUM PROGESTERONE LEVELS

The progesterone concentration in the peripheral blood of swamp and Murrah buffaloes is low at oestrus (Day 0, 0.13 - 0.27 ng/ml) and does not rise until after day 5. The first significant increase in progesterone concentration occurs about 7 days after estrus (Ahmed *et al.*, 1977). Values increase until day 14 - 16 with a lower mean value of 1.51 - 2.6 ng/ml in swamp buffalo (Kamonpatana *et al.*, 1979b; Jainuddin *et al.*, 1982; Kanai and Shimizu, 1984) compared to the values 4.00 - 4.26 ng/ml in Murrah buffaloes (Bachlaus *et al.*, 1980; Arora and Pandey, 1982a).

Plasma progesterone level directly reflected the function of corpus luteum, it was considered as an indicator of ovarian function and hence, it can be used as a marker to predict postpartum ovarian activity (Webb *et al.*, 1980).

Chauhan *et al.* (1982) studied the responses of PGF_{2α} in subestrus buffaloes. Serum progesterone levels were variable before treatment of PGF_{2α} (0.60 to 4.90 ng/ml). The levels of progesterone decreased abruptly within 24 hours following PGF_{2α} treatment and were minimum on day 2 to 5 or 6. Basal levels of progesterone were observed on day 13 to 18 with 2nd PGF_{2α} injection.

Several workers determined the blood progesterone level until first postpartum ovulation as less than 1.0 ng/ml of serum/plasma (Rajasundaram, 1994) whereas, Webb *et al.* (1980) reported it was more than 1.0 ng/ml

Hixon *et al.* (1981) found that in postpartum anestrous cows level of reported less than 1.0 ng/ml of plasma progesterone at the time of synchromate-B withdrawal in norgestomet treated dairy heifers.

Desilva *et al.* (1981) found that cows with plasma progesterone level of 0.6 ng/ml at 12 h after standing estrus had the lowest conception rate.

Takkar *et al.* (1982) observed serum progesterone level 0.360 ± 0.062 & 0.334 ± 0.066 , 3.372 ± 0.550 & 3.693 ± 0.378 and 0.321 ± 0.072 & 0.536 ± 0.066 ng/ml at estrus, 11 days after estrus and 21 days after estrus in normal cyclic buffalo heifers and cows, respectively. Pahwa and Pandey (1983) reported that it was 0.09 & 0.11, 2.41 & 2.46 and 0.31 & 0.54 ng/ml in non-pregnant primiparous and multiparous buffaloes respectively whereas; it was 0.10 & 0.12, 2.96 & 2.21 and 4.16 & 3.45 ng/ml in pregnant primiparous and multiparous buffaloes, respectively.

Gurdial singh *et.al.* (1983) treated summer anestrous buffaloes with PRID and observed that progesterone level of 0.42 ± 0.12 ng/ml before treatment and 0.261 ± 0.289 to 1.85 ± 0.598 ng/ml at PRID removal whereas, it reached 2.38 ± 0.438 and 0.436 ± 0.106 ng/ml in ovulated and anovulated animals respectively.

Hoagland and Barnes (1984) reported that the serum progesterone in the syncromate-B treated postpartum anestrous cows were initially inhibited and remained at the minimum level for the remainder of the implant period.

Troxel and Kesler (1984) treated postpartum anestrous cows with GnRH 24 h after implant removal and reported less than 0.5 ng/ml progesterone throughout the implant period. However, progesterone level elevated from day 2 and reached maximum of 2.0 ng/ml on day 8 after GnRH administration.

King *et.al.* (1988) reported less than 1.0 ng/ml progesterone level at 8 days after implant removal in 25 percent of the ovulated heifers.

Guridial Singh *et.al.* (1988b) reported serum progesterone level of 1.22 to 4.0 ng/ml on day 12 in ovulating animals whereas, anovulating animals registered basal values ranging between 0.1 and 0.6 ng/ml in norgestomet treated anestrus buffaloes.

Balasubramanian (1989) observed that the mean progesterone level at norgestomet induced estrus in fertile and non-fertile postpartum anestrus cows were 0.72 ± 0.22 and 0.94 ± 0.18 ng/ml, respectively.

Inderjeet *et.al.* (1996) recorded high progesterone level in norgestomet plus GnRH treated deep anestrous cows at 12 days after ovulatory heat.

Thompson *et.al.* (1999), observed serum progesterone concentration of 0.42, 0.75 and 0.33 ng/ml and 0.42, 1.08, and 0.33 ng/ml at d 0, d 7 and d 9 in anestrous

suckled beef cows treated with ovsynch and ovsynch plus norgestomet. They also reported that elevated levels of progesterone (>1 ng/ml) in 75% of anestrus cows within 7 days following a GnRH injection and a corresponding reduction in the incidence of short estrous cycles following PGF_{2α} induced luteolysis (7 days later).

Agarwal *et.al.* (2001) studied the progesterone profile in anestrus cattle treated with norgestomet, norgestomet + PMSG and norgestomet + PMSG + PGF_{2α}. The progesterone profiles (ng/ml) at insertion of implant in above three treatment groups were 0.496 ± 0.029 , 0.620 ± 0.043 and 0.498 ± 0.05 , at withdrawal of implant 0.888 ± 0.038 , 0.746 ± 0.027 and 1.110 ± 0.108 , at estrus 0.646 ± 0.034 , 0.556 ± 0.047 and 0.682 ± 0.096 , at 10 days post AI with CL 2.62 ± 0.024 , 2.62 ± 0.058 and 2.16 ± 0.05 and without CL 0.790 ± 0.16 , 0.570 ± 0.05 and 0.464 ± 0.06 , respectively.

Kavani *et.al.* (2005) studied the postpartum progesterone profile of fertile and infertile oestrus cycles in Surti buffaloes, the plasma profile revealed that the progesterone level was significantly higher in fertile than the infertile cycle on day 14 (2.14 ± 0.21 vs 1.20 ± 0.19 ng/ml) and day 21 (2.57 ± 0.21 vs 0.42 ± 0.04 ng/ml) but not at oestrus (0.37 ± 0.02 vs 0.39 ± 0.05 ng/ml) or day 7 (1.29 ± 0.08 vs 1.12 ± 0.11 ng/ml) post breeding. Moreover, the infertile buffaloes had a combined pattern of delayed rise and low level of progesterone indicative of insufficient luteal function.

Dugwekar *et.al.* (2008) studied the progesterone levels in different stages of estrous cycle in Jafarabadi buffaloes and recorded the serum progesterone levels of 0.55 ± 0.22 , 0.50 ± 0.14 , 2.90 ± 0.46 and 2.90 ± 0.10 ng/ml on day 1, 7, 14 and 21 of the estrous cycle. They also recorded the serum progesterone levels of 0.66 ± 0.33 , 0.88 ± 0.34 , 0.40 ± 0.19 , 0.71 ± 0.36 , 1.23 ± 0.50 , 0.51 ± 0.23 and 0.95 ± 0.40 ng/ml in 4, 5, 6, 7, 8, 9 and 10 months of postpartum.

Sharma *et.al.* (2008) reported that there was an inverse relationship between circulatory progesterone profile at the time of insemination and subsequent conception rate, the mean plasma progesterone concentration at fertile estrus was significantly lower than the infertile estrus (0.26 ± 0.02 vs 0.97 ± 0.13 ng/ml).

Ghuman *et.al.* (2009) studied the effect of ovsynch protocol in treatment of true anestrus buffalo heifers and recorded the plasma progesterone concentrations (ng/ml) of 0.16 ± 0.03 on day 0, 0.43 ± 0.10 on day 7 and 0.03 ± 0.01 ng/ml on day 9.

Mondal *et.al.* (2010) reported that peripheral plasma concentrations of progesterone ranged between 0.3 ± 0.06 to 1.94 ± 0.03 ng/ml during estrous cycle in buffalo. Plasma levels which were lowest (0.30 ± 0.06 ng/ml; $\text{Tau}^2 = 0.0125$) during perioestrus phase increased to 0.47 ± 0.07 ng/ml ($p = 0.067$) during early luteal phase and then further to 1.94 ± 0.03 ng/ml ($\text{Tau}^2 = 0$; $p < 0.001$) during mid luteal phase. The concentration declined thereafter to 1.24 ± 0.02 ng/ml ($\text{Tau}^2 = 0$) during late luteal phase.

Ghuman *et.al.* (2012) recorded plasma progesterone concentrations of 1.12 ± 0.24 , 0.73 ± 0.18 and 0.66 ± 0.22 ng/ml on day 0, 1.13 ± 0.28 , 2.21 ± 0.32 and 0.81 ± 0.18 ng/ml on day 7, 0.45 ± 0.17 , 0.15 ± 0.04 and 0.27 ± 0.09 ng/ml on day 9 and 0.35 ± 0.30 , 0.11 ± 0.04 and 0.13 ± 0.04 ng/ml on day 10 with GPG, GPG + CIDR and GP - hCG treatments in lactating anestrus buffaloes.

2.8.2 SERUM ESTROGEN LEVELS

The concentration of oestradiol-17 β in blood during the follicular phase of the estrous cycle also appears to be relatively less than that in cattle (Avenell *et al.*, 1985; Kanai *et al.*, 1990; Roy and Prakash, 2009). Although this has been suggested as a

possible reason for the lesser intensity of oestrus exhibited by buffalos. Studies on Italian buffalo have shown no differences in the endocrine profiles of those with overt and oestrus without an associated ovulation (Zicarelli, 1997a and Borghese, 2005).

Circulating estradiol concentrations remain low during the luteal phase with minor fluctuations (10 - 20 pg/ ml) around Days 4 and 10 of the estrous cycle in river buffalo (Batra and Pandey, 1982; Samad *et al.*, 1988) , but not in swamp buffalo (Kanai and Shimizu, 1984). Peak concentrations of estradiol (30 - 35 pg/ml) were detected on the day of estrus or one day before (Batra and Pandey, 1982) followed by a decline to 5–10 prostaglandins/ml within two days.

Singh and Madan (2000), estimated the plasma estrogen levels in different age groups of Murrah buffalo heifers and the concentrations (pg/ml) ranged from 21.26 ± 0.54 to 23.25 ± 0.74 , 20.69 ± 1.04 to 22.34 ± 0.66 and 21.41 ± 1.07 to 24.49 ± 0.80 in 12, 24 and 30 months old Murrah buffalo heifers, respectively.

Patel *et.al.* (2003) reported considerable elevation in circulating estradiol levels (up to 125.00 ± 21.83 pg/ml) after insertion of norgestomet ear implant and injection of estradiol valerate and progesterone levels fluctuated between 1.25 to 1.66 ng/ml on the day of estrus.

Dugwekar *et.al.* (2008) studied the estradiol levels in different stages of estrous cycle in Jafarabadi buffaloes and recorded the serum estradiol levels of 40.20 ± 19.68 , 2.07 ± 1.67 , 5.30 ± 2.02 and 8.66 ± 5.01 pg/ml on day 1, 7, 14 and 21 of the estrous cycle. They also recorded the serum estradiol levels of 12.73 ± 5.12 , 5.00 ± 2.54 , 5.22 ± 2.35 , 8.00 ± 3.92 , 8.37 ± 2.40 , 7.77 ± 4.01 and 7.73 ± 4.66 pg/ml in 4, 5, 6, 7, 8, 9 and 10 months of postpartum.

Mondal *et.al.* (2010) reported that plasma estradiol concentrations were maximum (22.48 ± 0.32 pg/ml; $\text{Tau}^2 = 15.93$) during perioestrus phase and declined ($p < 0.001$) to 11.04 ± 0.13 pg/ml ($\text{Tau}^2 = 0$) during mid luteal phase and increased to 12.29 ± 0.21 pg/ml ($\text{Tau}^2 = 0$; $p < 0.001$) during late luteal phase.

Ghuman *et.al.* (2012) recorded plasma estradiol concentrations of 415.8 ± 207.6 , 446.7 ± 172.5 and 456.9 ± 130.3 pg/ml on day 9 and 390.8 ± 174.9 , 478.1 ± 103.5 and 640.5 ± 129.0 pg /ml on day 10 with GPG, GPG + CIDR and GP-hCG treatments in lactating anestrous buffaloes .

TABLE. 1 STATEMENT SHOWING THE ESTRUS & OVULATION INDUCTION EFFICACY OF DOUBLE PG HORMONAL TREATMENT IN POSTPARTUMANOESTRUS BUFFALOES

S.No	Reference	Kind of Animal	Estrus response rate (%)	Interval to time of onset of estrus (hours)	Ovulation rate (%)	Conception rate at induced estrus(%)
1.	Chauhan <i>et al.</i> ,1981	Buffaloes	83.3	71.3	66.6	20.0
2.	Dhaliwal <i>et al.</i> , 1988	Buffaloes	85.5	----	92.85	
3	Pant and Singh,1991	Buffaloes	31.0 (BS)	69.3 (49-72)		54.8
4	Chohan <i>et al.</i> , 1998	buffaloes	87.8 (BS)	73.2		53.1
5	Srivastava <i>et al.</i> , 2005	Buffaloes	50.0 (Summer)		88.2%	40.0
6	Dadarwal <i>et al.</i> , 2009	Subestrus buffaloes	100	72- 96	100	50.0
7	Kumar <i>et al.</i> , 2013	Buffaloes	90			44.44

TABLE. 2 STATEMENT SHOWING THE ESTRUS AND OVULATION INDUCTION EFFICACY OF

OVSYNCH TREATMENT IN POSTPARTUM ANOESTRUS BUFFALOES

S.No	Reference	Kind of Animal	Estrus response rate (%)	Interval to time of onset of estrus (hrs)	Ovulation rate (%)	Conception rate at induced estrus(%)	Overall conception rates(%)
1.	Irikura <i>et al.</i> , 2003	Murrah heifers			81.81		18.18
2.	Camelo <i>et al.</i> , 2002						46.67% if AI 12h later 27.59% if AI 24h later
3.	Baruselli <i>et al.</i> , 2003	Buffaloes in Brazil			60.6		48.8
4.	Neglia <i>et al.</i> , 2003	Italian Mediterranean buffaloes	88				36
5.	De Rensis <i>et al.</i> , 2005	Italian Mediterranean buffaloes					35.7 in cyclic 4.7 in non cyclic
6.	Presicce <i>et al.</i> , 2005	Italian Mediterranean buffaloes			80 in BH 82 in BC		40 in BH 42.8 in BC
7.	Ravikumar <i>et al.</i> , 2005	Murrah buffaloes	41.66	48.80 ± 7.74h	83.33	33.33	----
8.	Ali & Fahmy, 2007	Egyptian buffaloes	----	----	80 (cyclic) 100 (acyclic)		60 (cyclic) 37.5 (acyclic)
9.	Ali <i>et al.</i> , 2008	Egyptian buffaloes			85.7		58.3
10.	Warriach <i>et al.</i> , 2008						36.3 in BS 30.4 in LBS
11.	Ghuman <i>et al.</i> , 2009	Murrah heifers	82		100	18	----
12.	Chaikhun <i>et al.</i> , 2010	Thai swamp buffaloes	100				51.4
13.	Malik <i>et al.</i> , 2010	Murrah buffaloes	87.5	1.29 ± 0.36 days	87.5	12.5	75
14.	Atanasov <i>et al.</i> , 2011	Bulgarian murrah buffaloes	73.3				40
15.	Malik <i>et al.</i> , 2011	Murrah buffaloes	40	< 2 days	80	20	60
16.	Azawi <i>et al.</i> , 2012	Iraqi northern buffaloes	35	74.8 ± 6.3	----	5	5
17.	Ghuman <i>et al.</i> , 2012					44.4	
18.	Jabeen <i>et al.</i> , 2012	Nili-Ravi buffaloes	87.5 in B.S 36.36 in N.B.S	48 hours after PG injection		----	----
19.	Derar <i>et al.</i> , 2012				100 -BH 88- BC		11.11 -BH 62.5 - BC
20.	Yotov <i>et al.</i> , 2012	Bulgarian Murrah buffaloes	66.7	----	55.6	----	38.8

**TABLE. 3 STATEMENT SHOWING THE ESTRUS AND OVULATION INDUCTION
EFFICACY OF NORGESTOMET IN POSTPARTUM ANESTROUS BUFFALOES**

S.No	Reference	Kind of Animal	Estrus response rate (%)	Interval to time of onset of estrus(hrs)	Ovulation rate (%)	Conception rate at induced estrus(%)	Overall conception rates(%)
1.	Shah <i>et al.</i> , 1987	Nili-Ravi buffaloes (True-anestrous)			70		53.3
2.	Usha Chhatry <i>et al.</i> , 1999	Murrah buffaloes	80 in B.S 60 in N.B.S	62.8 ± 1.04 in B.S 63.2 ± 8.7 in N.B.S	----	70 in B.S 30 in N.B.S	----
3.	Hattab S A <i>et al.</i> , 2000	Egyptian buffaloes	60	----	----	----	----
4.	Pant H.C <i>et al.</i> , 2002	Murrah buffaloes	100	36 to 48	44.4	11.1	----
5.	Markandeya & Bharkad, 2002	Murrah buffaloes	100	40.20 ± 2.78h	----	50	83.3
6.	Neglia <i>et al.</i> , 2003	Italian Mediterranean buffaloes	83				28.2
7.	Anand singh <i>et al.</i> , 2004.	Murrah buffaloes	87.5	2.142 ± 0.508 days after implant removal			
8.	Presicce <i>et al.</i> , 2005	Italian Mediterranean buffaloes	----	----	76.4	---	70.5
9.	Murugavel <i>et al.</i> , 2009	Murrah buffaloes	----	----	57.6	----	27.3
10.	Vivek Naik <i>et al.</i> , 2009		87.5	60.72 ± 17.72	----	----	71.42
11.	Malik <i>et al.</i> , 2010	Murrah buffaloes	100	2.86 ± 0.13 days	100	71.4	85.7
12.	Harendra Kumar, 2010	Murrah buffaloes	59.1	----	----		53.9
13.	Caesar <i>et al.</i> , 2011	Murrah buffaloes	71.4 to 100	72 ± 10.76	----	----	57.1 to 66.6
14.	Yotov <i>et al.</i> , 2012	Bulgarian Murrah buffaloes	94.4	----	72.2	----	55.6

TABLE.4 STATEMENT SHOWING THE ESTRUS & OVULATION INDUCTION EFFICACY OF NORGESTOMET PLUS PMSG HORMONAL TREATMENT IN LACTATING ANOESTRUS BUFFALOES

S.No	Reference	Kind of Animal	Estrus response rate (%) Breeding season	Interval to time of onset of estrus (hours)	Ovulation rate (%)	Conception rate at induced estrus (%)
1.	Rao <i>et al.</i> , 1982	Buffaloes	100 Breeding season	----		36
2.	Singh <i>et al.</i> , 1988	Buffaloes	88.0 (Summer)	----		50
3.	Agarwal <i>et al.</i> , 2001	Cows	71.42	55.5±2.87 hours		
4.	Anand Singh, 2004	Murrah buffaloes	87.5 Breeding season	3.42 days		-
5.	Presicca <i>et al.</i> , 2005	Buffaloes			88.2	
6.	Nayak <i>et al.</i> , 2009	Buffaloes	100 Breeding season	2.75 days		75
7.	Malik <i>et al.</i> , 2010	Murrah buffaloes	100 Breeding season	2.86±0.13 h		85.7
8.	Kumar <i>et al.</i> , 2010	Buffaloes	86.84	----		33.33
9.	Caesar, 2011	Buffaloes	100 Breeding season	65.14		85.7
10.	Mahesh <i>et al.</i> , 2011	Buffaloes	70.00	65.2 ± 16.5h after 2 nd GnRH inj		70
11.	Yadav <i>et al.</i> , 2001	Buffaloes	100	3.42 days	100	83.33

MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

Postpartum (70) parous lactating graded Murrah buffaloes (*Bubalus bubalis*) maintained under standard feeding and management at Buffalo research station, Vekataramannagudem (15⁰⁰ and 16¹⁰ North latitude and 79⁰⁴ and 80⁰² East Longitude), of Sri Venkateswara Veterinary University, Andhra Pradesh, India were included in this study. The present investigation was designed to treat anestrus (both subestrus and true anestrus) buffaloes using PGF_{2α}, GnRH, PMSG and Norgestomet for inducing and synchronizing ovulatory estrus in anestrus buffaloes during low breeding season. The study of estrus induction treatments were initiated during the months (low or towards the end of breeding season) of December 2013 to May 2014.

3.2 SELECTION OF BUFFALOES AND EXPERIMENTAL DESIGN

Seventy parous (1 to 6) Murrah buffaloes that calved normally from March to October 2013, aged between 6 - 10 years and weighing between 350 to 500 kg, that have not been detected in estrus since 3 to 9 months were monitored for ovarian activity and estrus pattern from the month of December 2013 to February 2014. Buffaloes which have not shown estrus during this period were confirmed as anestrus based on two rectal palpations, ultrasound scanning of ovaries performed at 10 day interval and further validated based on serum progesterone assay. The buffaloes, which have shown standing estrus subsequent to rectal palpation during the experimental period, have been inseminated and considered as control group (Plate.1).

Among these, seventy parous Murrah buffaloes which have not exhibited estrus with no palpable structures and buffalo cows with palpable structures on either of the ovaries with normal tubular genitalia and free from palpable genital abnormalities, confirmed as



I . Double prostaglandin protocol (Group - A)



II . Ovsynch protocol (Group - B)



III . CRESTAR + PMSG protocol (Group - C)

Plate. 1 Experimental animals for different protocols

trueanoestrus and subestrus buffaloes, respectively. The confirmation of true anoestrus and subestrus buffaloes was done based on transrectalultrasonography (TRUS)plus

blood sampling for progesterone (-10 days and day 0) performed at 10 days interval for presence or absence of corpus luteum. Blood samples were collected to estimate Progesterone (P4) and Estradiol profile at the time of initiation of treatment to correlate with ovarian activity. These parity buffaloes were utilized for investigation of hormonal estrus and ovulation induction.

3.3 ESTRUAL EVENTS IN ANESTRUS ANIMALS THAT HAVE EXHIBITED ESTRUS CONSEQUENT TO RECTAL EXAMINATION DURING THE EXPERIMENTAL PERIOD

3.3.1 ESTRUS DETECTION

Seventy lactating buffaloes were kept under observation for estrus detection using a teaser and visual observation at 6 hours interval from 6 AM to 6 PM in a day. Standing or observable behavioral estrus was considered to have occurred when the buffalo cow stood to be mounted. Estrus was confirmed by rectal examination and ultra sound scanning of reproductive tract and ovaries using portable ultra sound system with transrectal linear probe (7.5* MHz) at the time of insemination. Buffaloes were considered to be in estrus when the largest follicle showed slight fluctuation to touch (10 to 15 mm), the uterus showed very strong contractility (tonicity) and the external orifice of the cervix was open. Once, the estrus was confirmed, the estrus pattern parameters including the time interval from the time of rectal palpation to first standing estrus, conception rate, number of services per conception were recorded (Plate.2).

3.3.2 INTENSITY OF ESTRUS (ESTRUS BEHAVIOUR)

Intensity of estrus was classified as intense, intermediate and weak according to the method described by Rao and Rao (1981) with slight modification.

*PROSOUND 2 Hitachi Aloka Medical Ltd, Japan.

3.3.3 DURATION OF ESTRUS (HOURS)

Duration of behavioral estrus was estimated in hours from the time of first appearance (first acceptance of teaser) to cessation (refusal of teaser) of estrus signs in all the buffalo cows.

3.3.4 ARTIFICIAL INSEMINATION AT OBSERVED ESTRUS

All the buffaloes in standing estrus subsequent to rectal palpation were bred by artificial insemination using 0.25 ml frozen thawed semen at 8 to 12 hours after the onset of estrus. The buffaloes that did not conceive at first estrus were observed for subsequent estrus and inseminated. The conception rate at first A.I and subsequent inseminations was recorded.

Score card

		Points		
I	Behavioral changes			5
	1. Motor activity (Restlessness and alertness)	1		
	2. Mounting on other animals	1		
	3. Standing to be mounted	1		
	4. Posture	1		
	5. Chin resting and rubbing	1		
II				5
	1. Vulval oedema		2	
	a) Highly oedematous	2		
	b) Oedematous	1		
	c) Not oedematous	0		
	2. Urination		1	
	3. Genital discharge		2	
	a) Large volume, stringy	2		
	b) Moderate volume stringy to viscus	1		
c) Sparse volume viscus	0			
III				5
	1. Fern pattern			
	a) Typical fern pattern	2		
	b) Atypical fern pattern	1		
	c) No fern pattern	0		
	2. Cervical relaxation		1	
	3. Uterine tonicity		2	
	a) Highly tonic	2		
b) Tonic	1			
c) No tonic	0			
		15		

Intense : 10 - 15 points Intermediate : 5 - 10 points Weak : < 5 points

3.3.5 OVULATION RATE

The ovulation rate was calculated as number of animals that had corpus luteum on day 10 of the estrous cycle against the number of animals detected in estrus.

3.3.6 CONCEPTION RATE

Conception rate at induced estrus was calculated as percentage of buffaloes conceived at first and second inseminations and confirmed by transrectal ultrasonography on day 30 post A.I. Pregnancy was confirmed by palpation per rectum on day 60 post insemination.

3.3.7 OVERALL CONCEPTION RATE






Overall conception rate was calculated by percentage of buffaloes conceived, of the total buffaloes inseminated at first and second service

3.3.8 SERVICES PER CONCEPTION (A.I. INDEX)

The number of inseminations required per conception were recorded and expressed as AI index.

3.4 ULTRASOUND SCANNING OF UTERUS AND OVARIES ON ESTRUS DAY AND AT DAY 10

The reproductive tract and ovaries of estrus buffaloes, which have exhibited estrus consequent to rectal palpation were scanned to measure the diameter of ovulatory follicle size and the uterus to know the ultrasonographic appearance of the uterus thickness, evidence of edema and accumulation intrauterine fluids. The disappearance of ovulatory follicle and presence of corpus luteum 10 days after estrus considered as ovulation. The size of the ovulatory follicle, ovulation rate were presented.

		
<p>Lutalyse (PGF₂α) inj</p>	<p>Receptal (GnRH) inj</p>	<p>Folligon</p>
		
<p>Crestar ear implant + inj</p>	<p>Crestar insertion</p>	<p>Transrectal ultrasound</p>
		
<p>Xema Progesterone Kit</p>	<p>Xema Estradiol Kit</p>	<p>Serum vacutainers</p>
<p>Plate. 2 Hormones and instruments used in estrus and ovulation induction of Anestrus Buffalo cows</p>		

3.5 BLOOD SAMPLING IN POSTPARTUM ANESTRUS BUFFALOES

(n=10)

For progesterone and Estradiol estimation, blood samples were collected from 10 she buffaloes which have not exhibited estrus after rectal palpation and with no palpable corpus luteum or follicle confirmed by two ultra sound examinations from jugular vein with the help of serumvacutainers and disposable needles on day -10, day 0 for analysis of progesterone and estrogen to identify estrus status at initiation of treatment. The serum is separated by centrifuging at 3000 rpm for 15 minutes. The separated serum is stored at -20°C in plastic bullets until assay.

3.5.1 PROGESTERONE (P_4) ASSAY

The serum progesterone concentration was estimated using ELISA technique with the help of progesterone kits (PROGESTERONE EIA, XEMA Co.,Ltd,Russia) The Xema progesterone EIA is a 96 well plate, solid phase immunoassay, utilizing the competition enzyme immunoassay principle (Plate.2).

Progesterone present in the sample competes with enzyme labeled progesterone conjugate for binding with antiprogesterone antibody immobilized on the micro-well surface. The amount of conjugate that binds to the microwell surface will decrease in proportion to the concentration of the progesterone in the sample. The unbound sample and conjugate were then removed by washing and the color development reagents (substrate) are added. Upon exposure to the bound enzyme, a colour change will take place. The intensity of the color reflects the amount of bound enzyme progesterone conjugate and inversely proportional to the concentration of progesterone in the sample. The resulting colour was measured at 450 nm using a spectrophotometer. The sensitivity of the test was 0.1 ng/ml. Intra and inter assay coefficient of variations were

7.3 and 5.7% respectively. The results were presented by calculating the average absorbance value for each reference standard, control and sample. A standard curve was plotted with the known absorbance versus the corresponding concentrations of the standards on linear log-graph paper. The sample progesterone concentrations were determined from the standard curve using the percent absorbance of samples to be known. These results were used as supporting evidence for the ovarian activity observed per rectal examination during the study.

3.5.2 ESTROGEN (E2) ASSAY

The plasma estradiol concentration was estimated using ELISA technique with the help of estradiol kits (ESTRADIOL EIA, XEMA Co., Ltd, Russia). The Xema estradiol EIA is a 96 well plate, solid phase immunoassay, utilizing the competition enzyme immunoassay principle (Plate. 2).

Estradiol present in the sample competes with enzyme labeled estradiol conjugate for binding with antiestradiol antibody immobilized on the micro-well surface. The amount of conjugate that binds to the microwell surface will decrease in proportion to the concentration of the estradiol in the sample. The unbound sample and conjugate were then removed by washing and the color development reagents (substrate) are added. Upon exposure to the bound enzyme, a colour change will take place. The intensity of the color reflects the amount of bound enzyme estradiol conjugate and inversely proportional to the concentration of estradiol in the sample. The resulting colour was measured at 450 nm using a spectrophotometer. The sensitivity of the test was 13.6 pg/ml. Intra and inter assay coefficient of variations were 7.3 and 5.7% respectively. The results were presented by calculating the average absorbance value for each reference standard, control and sample. A standard curve was plotted with the

known absorbance versus the corresponding concentrations of the standards on linear log-graph paper. The sample estradiol concentrations were determined from the standard curve using the percent absorbance of samples to be known. These results were used as supporting evidence for the ovarian activity observed per rectal examination during the study.

3.6 ESTRUS AND OVULATION INDUCTION

The buffaloes that were confirmed as subestrus considered as Group A (n=16) treated with double PGF_{2α} (11 days apart) and the true anestrus buffaloes randomly divided into two groups assigned to Ovsynch (GPG) and norgestomet plus PMSG treatments and considered as Group B and Group C (n=16) (Plate.1) of pluriparous buffaloes. These treatments were initiated during the end of the (low) breeding season from December 2013 to May 2014 (Plate.1).

3.6.1 TREATMENT PROTOCOL FOR SUBESTRUS

3.6.1.1 Group A: Double PGF_{2α}

Sixteen subestrus lactating buffaloes were administered 25 mg of Dinoprost tromethamine (Lutalyse) on day 0 (treatment initiation). Eleven days later, buffalo cows were injected with second dose of 25 mg of Dinoprost tromethamine (Lutalyse), AI was performed at observed estrus (Plate. 2 & 3).

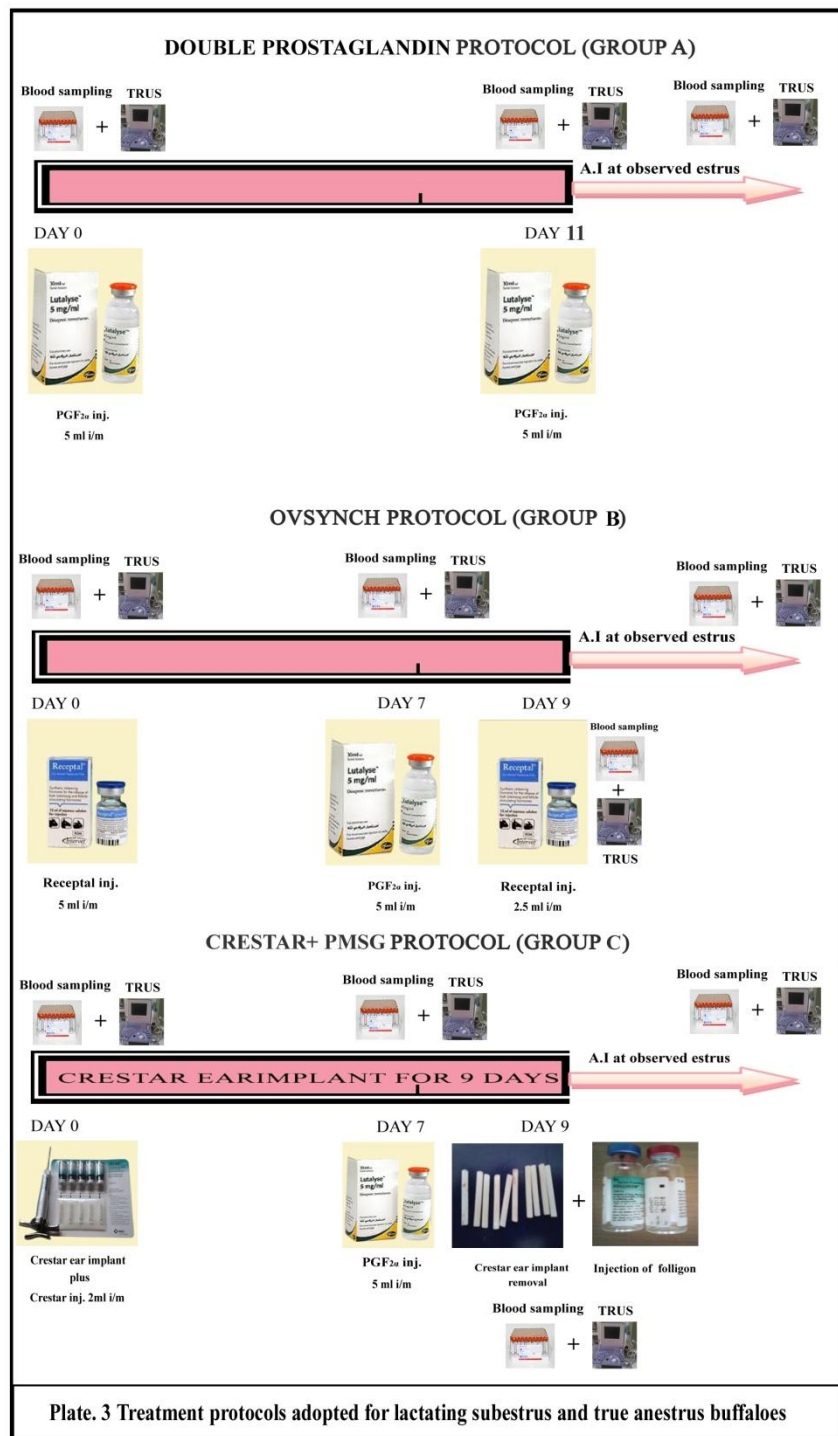
3.6.2 TREATMENT PROTOCOLS FOR TRUE ANOESTRUS

3.6.2.1 Group B: Ovsynch Protocol:

Sixteen trueanoestrus Buffaloes were administered 20µg of GnRH (Receptal) on day 0 (treatment initiation). Seven days later, buffalo cows were injected with 25 mg of PGF_{2α} (Lutalyse) and again 10 µg of GnRH (Receptal) on day 9 and AI was performed at FTAI (Plate. 2 & 3).

3.6.2.2 Group C: Crestar plus PMSG protocol:

Sixteen true anoestrus buffalo cows in this group were treated by inserting a Crestar ear implant containing 1.3 g of norgestomet. At the time of implant



insertion the buffaloes were administered 2ml of Crestar injection containing 3 mg of Norgestomet and 5 mg of estradiol valerate on day 0 (treatment initiation day) (Crestar, Intervet International, Netherlands), 25 mg of PGF 2α (Lutalyse, Pfizer) and 500 I.U of P.M.S.G (Folligon) was injected on day 7 and Crestar implant was removed on day 9 and AI was conducted at observed estrus (Plate. 2 &3).

Group D (Control): The buffaloes which have shown estrus subsequent to rectal palpation during the experimental period were bred at natural estrus and considered as control group (n=22).

Crestar insertion and precautions:

The Crestar applicator was washed in povidine solution (non-irritating antiseptic solution), then the tip of the applicator was dipped in liquid paraffin. The Crestar ear implant was inserted in the outer surface of the pinna of the ear to all the lactating true anestrus buffaloes of Group C.

Crestar retention rate

The rate of Crestar loss was recorded and presented in percent

3.7 BLOOD SAMPLING IN TREATMENT GROUPS (n=6)

In the postpartum buffalo cows (which did not show estrus up to 3 to 8 months postpartum), 10 days before (-10 days) the initiation of estrus and ovulation induction treatment, on the day of initiation of treatment (day 0) and on day 11 of the treatment for double PG (Group A), for the other two groups (Group B and Group C) on day -10, 0, 7 and on the estrus day blood samples were collected from jugular vein in serum vacutainers with disposable needles. Serum is separated by centrifugation at 1500-2000 rpm for 15 minutes. The separated plasma is stored at -20°C until assay.

Crestar: Intervet, Batch No. A097A02, Mfg:12/2010 Exp:11/2013

Lutalyse: Pfizer, Batch No. Z 00618 Mfg: 2/2012 Exp:01/2014.

Receptal: Intervet, Batch No.A285A01, Mfg:06/2010 Exp:05/2013
Folligon: Intervet, Batch No A036A01 Mfg:11/2011 Exp: 10/2014

3.7.1 Progesterone level in relation to fertility with Double PG, Ovsynch and Crestar plus PMSG treatments

The predictive physiological status of subestrus and true anestrus lactating buffaloes of Double PG, Ovsynch and Crestar plus PMSG treatments was determined by the concentration of progesterone in each of the blood samples collected treatment (day 0), and on day 11 of the treatment for double PG (Group A), for the other two groups (Group B and Group C) on day -10, 0, 7. A Concentration of progesterone $\geq 1\text{ng/ml}$ was defined as high (H) and indicative of presence of functional corpus luteum. A Concentration of progesterone $< 1\text{ng/ml}$ was defined as low (L) which indicated absence of functional corpus luteum. The two progesterone levels of double PG (Day 0 and day 11) and in other two groups (Ovsynch and Crestar plus PMSG) on d0, d7 and d9 were further divided into different possible permutations like, HH, HL, LL and LH; LLL, LLH, LHL, HHH, HHL, HLL and HLH.

3.8 ULTRA SOUND EXAMINATION IN TREATMENT GROUPS

Ovaries of buffaloes in each treatment group were monitored using portable ultra sound with transrectal linear array probe (7.5* MHz) on day -10 (10 days before treatment), on the day of treatment (day 0), on day 7, on day 9, on the day of induced estrus and on day 10 after A.I. Follicle size was categorized as small (3-5mm), medium (6-8mm), large (9-12mm), ovulatory (above 12mm) follicles and presence of corpus luteum. Ovulation was considered to occur when a large follicle that had been identified on the day of estrus and followed for presence of corpus luteum on day 10 after artificial insemination. All the inseminated non return she buffaloes were

examined on day 30 post A.I. and were considered positive when non echogenic fluid or echogenic embryo surrounded by anechoic fetal fluids observed. Palpation per rectum was done to confirm the pregnancy at day 45 - 60 post A.I.

3.9ESTRUS RESPONSE

The estrus response was defined as the percentage of treated buffalo cows that exhibited estrus symptoms. Detection of estrus over the first 6 days after the end of the treatment was taken as a response to the treatments.

3.9.1 ESTRUS PATTERN PARAMETERS

All the estrus pattern parameters as in the natural estrus were recorded viz., onset, intensity, duration of estrus, ovulation rate, estrous cycle length, conception rate at induced estrus, overall conception (conception at induced and subsequent estrus) and services per conception (A.I index).

3.9.2 OVULATORY RESPONSE

Ovulatory response was studied by rectal palpation and ultra sound scanning on day 10 after induced estrus, for the presence of corpus luteum and the results were expressed in percent of the total buffaloes treated. Ovulation was detected by sudden disappearance of a dominant follicle and confirmed by the development and presence of corpus luteum on day 10 by ultrasonographic examination.

3.9.3DEGREE OF SYNCHRONY OF ESTRUS AND OVULATION

The percentage of buffaloes, showed estrus and ovulation after the onset of estrus and within a peak period of 24 hours following the treatment, of the total buffaloes treated, considered as synchronous group.

3.9.4 COMPARISON OF ESTRUAL EVENTS AND CONCEPTION BETWEEN NATURAL AND TREATMENT GROUPS

The estrus response rate, time interval of onset of estrus, ovulation rate and conception rate of different treatment groups was compared.

3.9.5 COMPARISON OF REPRODUCTIVE PERFORMANCE AMONG TREATMENT GROUPS

The parameters studied in natural estrus and induced estrus was compared to know the advantages and disadvantages of induction of estrus in postpartum lactating cows.

3.10 STATISTICAL ANALYSIS

All the collected data was analyzed statistically as per the procedure described by Snedecor and Cochran (1989). In this study MTB statistical package was used for analysis of variance of different parameters.

RESULTS

Lactating anestrus graded Murrah buffaloes were treated using double PG (Group A) for subestrus and Ovsynch (Group B) and norgestomet plus PMSG (Group C) for true anestrus. Following these treatments, estrus induction, ovulation and fertility responses were observed and recorded. Lactating anestrus graded Murrah buffaloes which received no treatment served as control (Group D).

4.1. CLASSIFICATION OF ANESTRUS BASED ON TRANSRECTAL ULTRASONOGRAPHY (TRUS)

The distribution of true anestrus and subestrus based on TRUS findings were presented (Table. 5). In the present investigation 36 (51.4%) and 34 (48.6%) lactating buffaloes were diagnosed as true anestrus and subestrus buffaloes, respectively. Among the anestrus buffaloes, the ovaries bearing small follicles were 18/70 (25.7%), medium sized follicles 13/70 (18.5%), large follicles 3/70 (2.85%) and presence of corpus luteum was recorded in 34/70 (48.6%) lactating buffaloes (Plate. 4 and 5).

4.1.1 Incidence of estrus in anestrus buffaloes subsequent to rectal palpation

Out of 22 anestrus buffaloes kept as control, 8 buffaloes exhibited estrus during the experimental period. The details were presented along with treatment groups. The uterine change and follicular size was measured and presented (Plate. 6)



Plate.4 Sub estrus (Cyclic) lactating buffalo cows

- a) Ovary with a palpable corpus luteum
- b) Ovary with a palpable follicle (13mm)
- c) Ovary with a palpable follicle (14mm)

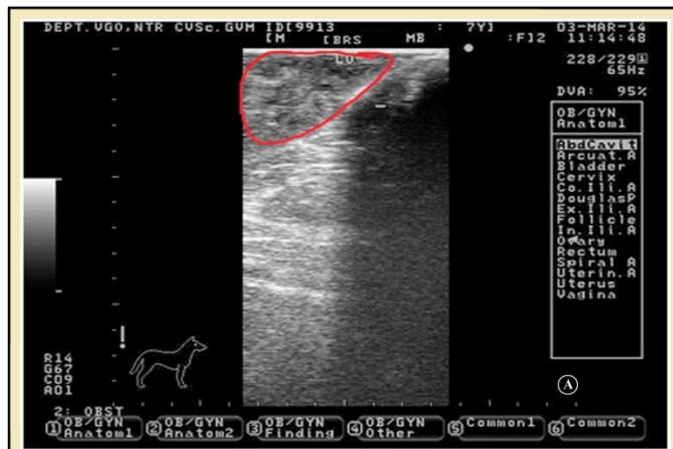


Plate.5 True anestrus (acyclic) lactating buffalo cows

a) Ovary with small follicles (>4-5mm)

b) Ovary with small follicles (< 4mm)



A. Echogenic uterine wall thickness



B. Anechoic Follicle measuring 1.3cm

Plate. 6 Ultrasonographic observations during estrus

4.2 PATTERN OF INDUCED ESTRUS IN LACTATING BUFFALOES

4.2.1 Estrus Detection

After second PGF_{2α} injection in group A, after PGF_{2α} injection on day 7 in group B (Ovsynch) and after implant removal in group C (Norgestomet plus PMSG) all the buffaloes were observed for estrus signs with the help of a teaser bull. Estrus detection was done by observing characteristic signs like

TABLE. 5 DISTRIBUTION OF ANESTRUS BUFFALOES BASED ON TRUS IMAGING OF OVARIES

PARAMETRS	TRUE ANESTRUS (%)	SUBESTRUS (%)
Small size follicles	18	-
Medium size follicles	13	-
Large follicles	3	-
Preovulatory follicles	2	-
Corpus luteum	0	34
Total	51.4(36/70)	48.6(34/70)

TRUS= Trans rectal ultrasonography

TABLE.6 ESTRUS DETECTION RATE OF INDUCED ESTRUS IN DOUBLE

PGF_{2α}, OVSYNCH AND CRESTAR PLUS PMSG IN LACTATING ANESTRUS BUFFALOES

Reproductive parameters	Treatment		
	Subestrus	True anestrus	
	GROUP A (Double PGF _{2α} 11 days apart)	GROUP B (OVSYNCH)	GROUP C (CRESTAR PLUS PMSG)
No. of animals treated	16	16	16
No. of animals in estrus	12	12	16
Estrus detection rate (%)	75	75	100

discharge especially while lying down, vulval edema, congestion of vestibular mucus membrane, frequent to intermittent urination, bellowing, restlessness and alertness, homosexual behavior like mounting and sniffing, standing to be mounted by teaser and its herdmates as described in the score card. Time interval from second PGF_{2α} injection in group A, after PGF_{2α} injection on day 7 in group B (Ovsynch) and after implant removal in group C (Norgestomet plus PMSG) to onset of estrus were measured.

4.2.2 ESTRUS RESPONSE

The estrus response following hormonal treatment was 75 (12/16), 75 (12/16) and 100 (16/16) per cent in A (Double PG), B (Ovsynch) and C (Crestar plus PMSG) groups respectively (Table. 6).

In group A out of 16 buffaloes administered with 1st PGF_{2α} eight buffaloes showed estrus signs during the treatment. This could be due to presence of corpus luteum and a large follicle (>10mm) in the subestrus buffaloes. In group B out of 16 buffaloes administered with first GnRH four buffaloes showed estrus signs. None of the buffaloes exhibited visible estrus signs during the treatment period in treatment group C (Plate. 7).

4.2.3 ONSET OF ESTRUS

Mean time to onset of estrus was, 61.39 ± 10.0 (48 to 96), 52.00 ± 170 (48 to 60) and 57.17 ± 2.09 (48 to 72) hours in group A (Double PG), B (Ovsynch) and C (Crestar plus PMSG), respectively. Analysis of variance of onset of estrus revealed that there was no significant difference ($P>0.05$) among the treatment groups (Table. 7).

4.2.4 DURATION OF ESTRUS

Mean estrus duration was 22.08 ± 0.96 (19 to 32) h, 16.83 ± 0.69 (14 to 22) h and 24.33 ± 1.33 (18 to 34) h in group A (Double PG), B (Ovsynch) and C (Crestar plus PMSG), respectively. Analysis of variance of duration of estrus showed nonsignificant difference ($P<0.05$) among Group A, B and C (Table. 7).

TABLE. 7 TIME INTERVAL TO ONSET OF ESTRUS AND INTENSITY OF INDUCED ESTRUS IN DOUBLE PGF_{2α}, OVSYNCH AND CRESTAR

PLUS PMSG IN LACTATING ANESTRUS BUFFALOES

Reproductive parameters	Treatment			
	Subestrus	True anestrus		Anestrus
	GROUP A (Double PGF _{2α} 11 days apart)	GROUP B (OVSYNCH)	GROUP C (CRESTAR PLUS PMSG)	Control D Consequent to Rectal palpation
Onset of estrus				
Mean ± SE (h) (Range)	61.39 ± 10.0 (48 to 96)	52.00 ± 1.70 (48 to 60)	57.17 ± 2.09 (48 to 72)	-
Intensity of estrus at the time of A.I.				
Intense	8.33% (1/12)	0	37.50% (6/16)	8.33% (1/12)
Intermediate	66.67% (8/12)	50.00% (6/12)	50.00% (8/16)	41.67% (5/12)
Weak	25.00% (3/12)	50.00% (6/12)	12.50% (2/16)	50.00% (6/12)
Duration of estrus (h)	22.08 ± 0.96 ^a (19 to 32)	16.83 ± 0.69 ^b (14 to 22)	24.33 ± 1.33 ^a (18 to 34)	21.67 ± 3.9 ^a (18 to 30)
Follicle size (mm) Mean ± SE (Range)	11.8 ± 1.02 ^a (9 to 13)	12.1 ± 2.01 ^a (9 to 14)	11.77 ± 1.77 ^a (10 to 13)	11.6 ± 1.62 ^a (10 to 13)

(Mean with different superscripts in a row differ significantly, P < 0.05)

**TABLE. 7a ESTRUS INTENSITY IN RELATION TO FERTILITY WITH DOUBLE PG,
OVSYNCH AND CRESTAR AND PLUS PMSG TREATMENTS**

Group	Animals Showed Intense estrus (%)	CR (%)	Animals showed Intermediate estrus (%)	CR (%)	Animals showed Weak estrus (%)	CR (%)
A (Double PG)	1/12 (8.33%)	1/1 (100%)	8/12 (66.67%)	7/8 (87.50%)	3/12 (25%)	2/3 (66.67%)
B (Ovsynch)	0/0	0/0	6/12 (50%)	5/6 (83.33%)	6/12 (50%)	6/6 (100%)
C (Crestar plus PMSG)	6/16 (37.50%)	4/6 (66.67%)	8/16 (50%)	7/8 (87.50%)	2/16 (12.50%)	2/2 (100%)

CR - Conception Rate.

4.2.5 INTENSITY OF ESTRUS

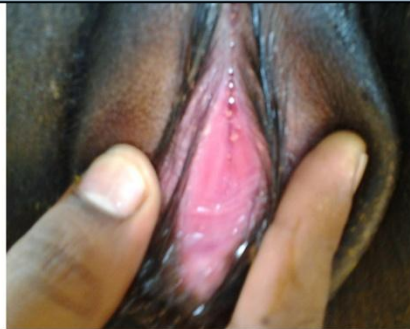
In the treatment groups Double PG (Group A), Ovsynch (Group B) and Crestar plus PMSG (Group C), intense to normal estrus with mucus discharge and behavioral signs was observed. The per cent of cows that showed intense, intermediate and weak estrus were 8.33, 66.66 and 25.00 in double PG group (A), 0, 50.00 and 50.00 in ovsynch group (B) and 37.50, 50.00 and 12.50 in Crestar plus PMSG group (C), respectively (Table. 7 and Plate. 7). Two (2/12) buffaloes in the control group have shown normal estrus during the treatment period. The effect of intensity of estrus on conception was presented in the Table. 7a.



Teaser bull identifying the induced estrus buffalo



Stringy cervical mucus discharge hanging from vulva



Congested vestibular mucus membrane

Plate. 7 Estrus response in estrus induced buffalo cows

4.2.6 DEGREE OF SYNCHRONY

Peak synchrony of 58.33, 66.67 and 62.50 per cent was observed between 48 to 72 hours after second PGF_{2α} injection in group A, after PGF_{2α} injection on day 7 in group B (Ovsynch) and after implant removal in group C (Norgestomet plus PMSG) (Table. 8).

4.3 ARTIFICIAL INSEMINATION SUBMISSION RATE

All the estrus induced buffaloes that were observed estrus in all the treatment groups were inseminated. The artificial insemination submission rate was 75.00, 75.00, 100 and 36.36 per cent in Double PG (Group A), Ovsynch (Group B), Crestar plus PMSG (Group C) and control (Group D) respectively were presented in Table. 9.

4.4 FERTILITY RESPONSE

4.4.1 OVULATORY RESPONSE

The ovulatory response was calculated as percentage of animals that ovulated over the total animals exhibited estrus. The ovulatory response in Double PG (Group A), Ovsynch (Group B), Crestar plus PMSG (Group C) and control (Group D) were presented in Table.10. An ovulatory response of 83.5 (10/12), 91.66 (11/12), 87.5(14/16) and 75.00 (6/8) per cent was observed in Group A, B, C and D, respectively. The size of the preovulatory follicle and corpus luteum in different treatment groups and in natural estrus was presented (Table. 10 and Plate. 8).



Plate.8 Preovulatory follicles on the estrus day in different treatments.

- a) Preovulatory follicle (10mm) in double PG
- b) Preovulatory follicle (13mm) in Ovsynch
- c) Preovulatory follicle (14mm) in Norgestomet plus PMSG

4.4.2 CONCEPTION RATE AT INDUCED ESTRUS

The conception rate was calculated as percentage of animals that were conceived to insemination at detected estrus at induced estrus in each group. The conception rates in buffaloes of various treatment groups are given in Table. 9. The conception rates were 58.33 (7/12), 66.66 (8/12), 62.5 (10/16) and 40.0 (2/8) conception in Double PG (Group A), Ovsynch (Group B), Crestar plus PMSG (Group C) and control (Group D) respectively.

4.4.3 OVERALL CONCEPTION RATE

The overall conception rate in the subestrus lactating buffaloes treated with Double PG (Group A) was 83.33 (10/12) per cent. In true anestrous buffaloes treated with Ovsynch (Group B) and Crestar plus PMSG (Group C) were 91.6 (11/12) and 81.25 (13/16) per cent, respectively. The conception rate was calculated as the percentage of animals that were conceived to the total number of animals inseminated as detected by TRUS at day 30 post AI (Table. 9 and Plate. 9). The buffaloes which had exhibited irregular estrus following second service were not included in this study.

**TABLE.8 SYNCHRONY OF ESTRUS IN TREATMENTS DOUBLE PGF_{2α},
OVSYNCH
AND CRESTAR PLUS PMSG IN LACTATING ANESTRUS BUFFALOES**

Reproductive parameters	Treatment			
	Subestrus	True anestrus		Anestrus
	GROUP A (Double PGF _{2α} 11 days apart)	GROUP B (OVSYNCH)	GROUP C (CRESTAR PLUS PMSG)	Control D Consequent to Rectal palpation
No. Of animals treated	16	16	16	22
No. Of animals exhibited estrus	12/16	12/16	16/16	8/22
Degree of synchrony No. Of animals in estrus (hours after treatment)				
0-24	0	--	--	12.50% (1/8) (With in 10 days)
24-48	16.67 % (2/12)	33.33% (4/12)	18.75% (3/16)	25.00% (2/8) (With in 20 days)
48-72	58.33 % (7/12)	66.67% (8/12)	62.50 (10/16)	62.50% (5/8) (Above20days)
72-96	25.00 % (3/12)	--	18.75% (3/16)	---



A . Day 25



B . Day 38



C . Day 45

Plate.9 Ultrasonographic monitoring of pregnancy diagnosis

TABLE. 9 ARTIFICIAL INSEMINATION SUBMISSION, CONCEPTION AND

PREGNANCY RATE IN DOUBLE PGF_{2α}, OVSYNCH AND CRESTAR PLUS PMSG IN LACTATING ANESTRUS BUFFALOES

Reproductive parameters	Treatment			
	Subestrus	True anestrous		
	GROUP A (Double PGF _{2α} 11 days apart)	GROUP B (OVSYNCH)	GROUP C (CRESTAR PLUS PMSG)	GROUP D (CONTROL)
Onset of estrus				
Artificial submission rate (%)	75 (12/16)	75 (12/16)	100 (16/16)	36.38 (8/22)
First service conception rate at induced estrus (%)	58.33 (7/12)	66.66 (8/12)	62.5 (10/16)	37.5 (3/8)
Second service conception rate at subsequent estrus (%)	40.00 (2/5)	25.00 (1/4)	50.00 (3/6)	40.00 (2/5)
Over all conception rate (%) at 30 days (TRUS)	75.00 (9/12)	75.00 (9/12)	81.25(13/16)	62.5 (5/8)
Pregnancy rate (%) at 45-60 days by rectal examination	66.66 (8/12)	66.66 (8/12)	81.25(13/16)	62.5 (5/8)

TABLE.10 OVULATORY RESPONSE IN DOUBLE PGF_{2α}, OVSYNCH AND

**CRESTAR PLUS PMSG IN LACTATING ANESTRUS
BUFFALOES**

Reproductive parameters	Treatment			
	Sub estrus	True anestrus		Control
	GROUP A (Double PGF _{2α} 11 days apart)	GROUP B (OVSYNCH)	GROUP C (CRESTAR PLUS PMSG)	GROUP D
Ovulatory response (%)	83.3 (10/12)	91.6 (11/12)	87.5 (14/16)	62.5(5/8)
Size of the CL on day 10 post A.I.(mm)	14.51±0.08 (12-16)	16.24±2.1 (11-18)	16.14±1.10 (12-18)	15.12±1.12 (13-17)

4.5 SERUM HORMONAL PROFILE

4.5.1 PROGESTERONE PROFILE

Serum progesterone level was estimated 10 d before implant insertion (-10 d) and on the day of treatment (d 0) to classify the anestrus. The Serum progesterone level on the day of second PGF_{2α} injection in group A, on day 7 before PG injection in group B, on day 7 before PG injection and at Crestar ear implant removal in group C and at induced estrus, 10 d post-AI in all the treatment groups, presented in the Table. 11.

The mean progesterone concentration 10 days before initiation of treatments in subestrus lactating buffaloes was 1.28 ± 0.17 (0.4 to 2.1) and on the treatment initiation day was 1.30 ± 0.11 (0.7 to 2.2) ng /ml. The mean progesterone concentration on the day of second PGF_{2α} injection on day 11 in group A (Double PG) was 1.34 ± 0.14 (0.9 to 2.1) and on the estrus was 0.72 ± 0.08 (0.3 to 1.2) ng/ml (Table. 11).

TABLE.11 MEAN LEVELS OF PROGESTERONE AND ESTRADIOL BEFORE AND AFTER TREATMENT IN DOUBLE PGF_{2α} TREATED LACTATING SUBESTRUS BUFFALOES (n=6)

Parameters	Treatment Group A Double PGF _{2α} (11 days apart)			
	Before treatment		After treatment	
	-10 days	On the day of treatment (1 st PG inj.)	On the day of treatment (2 nd PG inj.)	Estrus day
Progesterone (ng/ml)	1.28 ± 0.17 ^a (0.4 to 2.1)	1.30 ± 0.11 ^a (0.7 to 2.2)	1.34 ± 0.14 ^a (0.9 to 2.1)	0.72 ± 0.08 ^b (0.3 to 1.2)
Estradiol (pg/ml)	10.80 ± 1.21 ^a (6.0 to 16.0)	10.60 ± 1.13 ^a (7.0 to 16.0)	-	35.9 ± 1.13 ^b (18.0 to 42.0)

Means bearing different superscripts within the row differ significantly

The mean progesterone concentration 10 days before initiation of treatments in true anestrus lactating buffaloes was 0.80 ± 0.11 (0.30 to 1.4) and on the treatment initiation day, 0.85 ± 0.11 (0.40 to 1.5) ng/ml (Table. 12).

In treatment group B (Ovsynch) on day 7 at the time of PGF_{2α} injection mean progesterone concentration was 1.95 ± 0.98 (0.9 to 1.8) and on the estrus day was 0.60 ± 0.79 (0.4 to 1.5) ng/ml. The mean progesterone concentration on day 0 and day 7 of the treatment was significantly different with the progesterone concentration of estrus day ($P < 0.05$) (Table. 12).

In treatment group C (Crestar plus PMSG) the mean progesterone concentration on day 7 at the time of PGF_{2α} injection and on day the day of estrus was 2.03 ± 0.21 (1.4 to 3.2), 0.63 ± 0.07 (0.40 to 1.0) ng/ml respectively significant difference ($P < 0.05$). The mean P₄ concentration on day 10 after AI were 1.83 ± 0.29 (1.1 to 2.8), 1.91 ± 0.27 (1.1 to 2.62) ng/ml respectively in group A and C (Table. 11).

4.5.2 ESTRADIOL PROFILE

Serum estradiol levels were estimated on -10 d, 0 d (on the day of treatment) and on the estrus day in all the treatment groups,

The mean estradiol concentration 10 days before initiation of treatments were 10.80 ± 1.21 (6.0 to 16.0), 12.0 ± 1.71 (6.0 to 22.0) and 12.80 ± 1.66 (7 to 22.0) pg/ml in A, B and C groups respectively. Further, the mean estradiol concentrations on initiation of treatment were 10.60 ± 1.13 (7 to 16.0), 10.86 ± 1.52 (6.0 to 22.0) and 13.40 ± 1.59 (9.0 to 20.0) pg / ml in A, B and C groups respectively. The mean serum estradiol concentrations ten days before hormonal treatment and on initiation of treatment day were significantly not different ($P > 0.05$) (Table. 11 and 12).

The mean serum estradiol concentrations on the day of induced estrus in group A (double PG), group B (Ovsynch) and groups and C (Crestar plus PMSG) were 35.9 ± 1.13 (18 to 42), 37.91 ± 3.66 (25 to 43) and 42.3 ± 2.90 (26.0 to 52.0) pg/ml respectively with significant difference ($P < 0.05$). Though numerically there was difference in estradiol concentration but there is no significant difference among the treatment groups ($P > 0.05$).

The mean serum estradiol concentrations on the day of induced estrus was significantly different ($P < 0.05$) from serum estradiol concentration on the day of treatment initiation day (Table. 12).

TABLE.12 MEAN LEVELS OF PROGESTERONE AND ESTRADIOL BEFORE AND AFTERTREATMENT IN OVSYNCH AND CRESTAR PLUS PMSG TREATED LACTATING TRUE ANESTRUS LACTATING BUFFALOES (n=6)

Parameters	Treatment							
	GROUP B (Ovsynch)				GROUP C (Crestar plus PMSG)			
	BT		AT		BT		AT	
	-10 days	On the day of treatment (1 st GnRH inj.)	On day 7 At PGF _{2α}	On Estrus day	-10 days	On the day of treatment (at Crestar insertion)	On day 7 At PGF _{2α}	On Estrus day
Progesterone (ng/ml)	0.80 ±0.11 ^a (0.3 to 1.4)	0.85 ±0.11 ^a (0.4 to 1.5)	1.95 ±0.98 ^b (0.9 to 1.8)	0.60 ±0.70 ^a (0.4 to 1.5)	0.93±0.12 ^a (0.4 to1.2)	0.72±0.08 ^a (0.6 to1.2)	2.03±0.21 ^b (1.4 to 3.2)	0.63±0.07 ^a (0.4 to1.0)
Estradiol (pg/ml)	12.0±1.71 ^a (6.0 to 22.0)	10.86±1.52 ^a (6.0 to20.0)	19.0±0.83 ^b (16.0 to 24.0)	37.91±3.66 ^c (25.0 to 43.0)	12.80±1.66 ^a (7.0 to 22.0)	13.40±1.59 ^a (9.0 to 20.0)	28.00±2.91 ^b (14.0 to 42.0)	42.30±2.90 ^c (26.0 to52.0)

Means bearing different superscripts within the row differ significantly (P<0.05)

BT= BEFORE TREATMENT AT= AFTER REATMENT

4.6 Ovarian status assessment by serum progesterone and estradiol concentration in induced estrus and ovulation and its influence on estrus induction and conception rate at first service

4.6.1 SERUM PROGESTERONE

The incidence of cyclicity and conception rate of subestrus lactating buffaloes based on progesterone in each of the serum samples collected on d 0, d 11 in group A is presented (Table. 17). The (16) buffaloes in this group were classified as buffaloes with high (H), low (L) progesterone into different combinations based on the serum progesterone levels Viz., HH, HL, LH and LL. The HH and LH indicating high progesterone and HL low progesterone on the day of second PG (Table. 13).

In the present study, 8/16, 4/16 and 4/16 buffaloes included in group A (Double PG), had HH, LH and HL progesterone. The buffaloes that had HH had corpus luteum on both the occasions and HL, LH indicated presence of CL on day 0 and on the day 11 before second PG injection, as detected by TRUS. In the present study, in group A the estrus induction rate and conception rate at induced (first) estrus was 12/16 (75%) and 7/12 (58.33%) lactating buffaloes. The serum progesterone (0.72 ± 0.08) ng/ml level on the day of induced estrus was low and correlated with presence of preovulatory follicle. The buffaloes that had 1ng/ml progesterone had failed to conceive (Table. 13).

In the present study 0/16, 2/16, 9/16 and 3/16; 1/16, 0/16, 13/16 and 2/16 true anestrous buffaloes included in group B and C had HHH, HHL, HLL, LLL, LHH and LH L and LLH levels of progesterone in each of the serum samples collected on d 0, d 7 and d 9 (second GnRH in group B and Crestar removal in group C) respectively. The incidence of estrus and first service conception rate of true anestrous lactating buffaloes group B (Ovsynch) and C (Crestar plus PMSG) was 12/16, 16/16 and 50.00, 62.50 per cent respectively (Table. 13).

TABLE. 13 PROGESTERONE LEVEL IN RELATION TO FERTILITY WITH DOUBLE PG, OVSYNCH AND CRESTAR PLUS PMSG TREATMENTS

Progesterone	Physiological status	Subestrus		Progesterone	Physiological status	True anestrus					
		Double PG				Ovsynch		Crestar plus PMSG			
		Estrus response rate % (No/No)	Conception rate % (No/No)			Estrus response rate % (No/No)	Conception rate % (No/No)	Progesterone	Physiological status	Estrus response rate % (No/No)	Conception rate % (No/No)
D 0 and D 11 High P4 on day 11 at the time of PG				D 0 and D 7 High P4 on day 7 at the time of PG				D0,D7 and D9 High P4 on day 9 at Crestar removal			
High progesterone											
HH	No luteolysis	3/16	2/16	HH	Luteinization	0/16	0/16	LHL	Luteinization	0/16	0
LH	Ovulation after 1 st PG	5/16	3/16	LH	Ovulation after 1 st PG	8/16	6/16	LLH	Luteinization	8/16	4/16
HL	Synchronous cyclicity	4/16	2/16	HL	Synchronous cyclicity	2/16	2/16	LHH	Synchronous cyclicity	8/16	6/16
Total		12/16	7/16	Total		10/16	8/16	Total		16/16	10/16
Low progesterone											
LL	ANESTRUS	0/4	0/4	LL	ANESTRUS	0/6	0/6	LLL	ANESTRUS	0/0	0/0
Total		0	0	Total		0	0	Total		0	0

4.6.2 INFLUENCE OF SERUM PROGESTERONE ON ESTRUS INDUCTION AND CONCEPTION

The subestrus buffaloes which had high Serum progesterone concentration at the beginning of the treatment (day0) and on day 11 at the time of second PG induced to estrus (12/16) and conceived (7/12) at first service.

The true anestrous buffaloes which have shown elevated progesterone, LLH (8/16) (8/16), LHH (4/16) in Ovsynch (group B) and LLH (9/16), LHH (7/16) in group C (Crestar plus PMSG) 12/16, 16/16 buffaloes exhibited estrus and 8/12, 10/16 conceived at first (induced) estrus (Table. 13).

4.7 OVARIAN ACTIVITY IN TREATMENT GROUPS

The ovarian activity in subestrus buffaloes treated with double PG (group A) was classified as SF, MF, LF, PF, CL; on day 0, day 11 and on estrus day. Out of 16 subestrus buffaloes, 14/16 had MF, CL on day 0, 12/16 had LF, CL on day 11 and 12/16 had PF on the day of estrus day (Table. 14).

The ovarian activity in true anestrous buffaloes treated with Ovsynch was classified as SF, MF, LF, PF, CL; on day 0, day 7, day 9 and on estrus day. Out of 16 True anestrous buffaloes, 5/16 had SF, 11/16 had SFMF on day, 12/16 had MF, LF on day 7, 13/16 had LF, PF on the day9 and 15/16 had LFPPF on estrus day (Table. 14).

The ovarian activity in true anestrous buffaloes treated with Crestar plus PMSG (group C) was classified as SF, MF, LF, PF, CL; on day 0, day 7, day 9 and on estrus day. out of 16 True anestrous buffaloes, 5/16 had SF, 11/16 had SFMF on day 0, 12/16 had MF, LF on day 7, 13/16 had LF, PF on the day9 and 15/16 had LFPPF on estrus day.

TABLE. 14 OVARIAN STATUS IN RELATION TO FERTILITY WITH.DOUBLE PG, OVSYNCH AND CRESTAR PLUS PMSG TREATMENTS

Ovarian status	Physiological status	Subestrus		Progesterone	Physiological status	True anestrus					
		Double PG				Ovsynch		Progesterone	Physiological status	Crestar plus PMSG	
		Estrus response rate % (No/No)	Conception rate % (No/No)			Estrus response rate % (No/No)	Conception rate % (No/No)			Estrus response rate % (No/No)	Conception rate % (No/No)
D 0 and D 11				D 0 and D 7				D0, D7 and D9			
Type of palpable structure											
SF, MF,	Acyclic	0/16	0/16	SF,MF,	Acyclic	0/16	0/16	SF, MF, LF	Acyclic	0/16	0
LF, PF,	Could be cyclic	0/16	0/16	LF,PF,	Growth of follicle	4/16	2/16	MF, LF, PF,	Growth of follicle	8/16	4/16
PF, CL	cyclicit y	6/16	4/16	PF,CL	cyclicit y	4/16	4/16	LF, PF, CL	cyclicit y	8/16	6/16
CL, PF	cyclicit y	6/16	3/16	CL,PF	cyclicit y	2/16	2/16	PFCL, PF	cyclicit y		
Total		12/16	7/16	Total		10/16	8/16	Total		16/16	10/16
Low progesterone											
CL MF	ANESTRUS	0/4	0/4	MF,LF	ANESTRUS	0/6	0/6	MF,LF	ANESTRUS	0/0	0/0
Total		0	0	Total		0	0	Total		0	0

Small follicle (SF), Medium follicle (MF), Large follicle(LF), Preovulatory follicle (PF) and Corpus luteum (CL)

4.8 COMPARISON OF ESTRUAL EVENTS AND CONCEPTION BETWEEN NORMAL AND TREATED GROUPS

The estrus response, time interval to onset of estrus, ovulation rate and conception rate of different treatment groups were presented (Table. 15).

TABLE.15 ESTRUS DETECTION RATE, FERTILITY RESPONSE RATE IN TREATMENT AND CONTROL GROUPS

GROUP PARAMETERS	GROUP A (Double PG)	GROUP B (Ovsynch)	GROUP C (Crestar plus PMSG)	CONTROL GROUP D
No. of animals treated	16	16	16	22
No. of animals detected estrus	12	12	16	8
Interval to onset of estrus	61.39 ± 10.0 (48 to 96) ^a	52.00 ± 1.70 (48 to 60) ^a	57.17 ± 2.09 (48 to 72) ^a	➤ 20 days
A.I submission rate	75 (12/16)	75 (12/16)	100 (16/16)	36.36(8/22)
Ovulatory response at induced / natural estrus	83.3 (10/12)	91.6 (11/12)	87.5 (14/116)	62.5(5/8)
First service conception rate at induced estrus (%)	58.33 (7/12)	66.66 (8/12)	62.5 (10/16)	37.5 (3/8)
Second service conception rate at subsequent estrus (%)	40.00 (2/5)	25.00 (1/4)	50,00 (3/6)	40.00 (2/5)
Over all conception rate (%) at 30 days (TRUS)	75.00 (9/12)	75.00 (9/12)	81.25(13/16)	62.5 (5/8)
AI Index (No. Of services per conception)	1.8	1.7	1.6	2.6

CHAPTER-V

DISCUSSION

The present study entitled “**Treatment of anestrous graded Murrah buffaloes (*Bubalus bubalis*) using certain hormonal combinations by monitoring ovarian activity**” was intended for treatment of subestrus and true anestrous lactating buffaloes. In this study the efficacy of three different estrus synchronization treatments using Double PGF_{2α} (Group A) for treating subestrus buffaloes, Ovsynch (Group B) and Norgestomet plus PMSG (Group C) for treating true anestrous buffaloes was investigated during the low breeding season (from the month of December 2013 to May 2014) in the treatment of postpartum lactating anestrous Murrah buffaloes. Following estrus induction, ovulation and fertility responses were observed and recorded.

5.1 INCIDENCE OF TRUEANESTRUS AND SUB ESTRUS

Postpartum ovarian quiescence either due to true anestrous or subestrus constitutes the major reproductive failure in buffaloes (Shah *et al.*, 1990)

In the present investigation, critical differential diagnosis of the buffaloes that failed to exhibit estrus beyond 180 to 270 days postpartum using trans rectal ultrasonography revealed incidence of 51.4 (36/70) and 48.6 (34/70) per cent of true anestrus and subestrus, respectively.

The incidence of 51.4 per cent of true anestrous observed during low breeding season in this study is falling in line with findings of Hedao *et al.* (2008), who reported the overall percentage of non cyclic buffaloes as 59.70 per cent in summer season. Similar findings were also reported by Chauhan *et al.* (1979) who recorded 46.6 per cent. These differences in incidence of anestrus were attributed to variation in the environmental condition, managemental practices and nutritional status.

The 48.6 per cent of silent heat or subestrus occurred in this study is corroborating the findings of Sane *et al.* (1967) and Shah *et al.* (1990) in milch buffaloes with 6 - 73 per cent incidence reported under small farm conditions. The number of subestrus buffaloes were highest (32.6 per cent) during the autumn (Chauhan *et al.*, 1984) followed by the winter (28.4 per cent).

Ovarian inactivity is more frequent in buffaloes on low level of feeding than in those giving high level and also in summer than other seasons (Rao and Sreemannarayana, 1982; Chauhan *et al.*, 1984). The postpartum anestrus when extended to six months or more is known as prolonged anestrus or up to one year known as dead anestrus (Roberts, 1971). Many workers have reported very high incidence of anestrus in various breeds of buffaloes in field condition or at organized dairy farms (Luktuke and Sharma, 1978, Janakiraman, 1988 and Banerjee Roychowdary, 1992).

The per cent incidence of postpartum anestrus in buffaloes recorded by different states varied from 8.9 (Nasir Hussian Shah *et al.*, 1990); 14.9 (Luktuke *et al.*, 1973); 17 (Narinder Singh *et al.*, 1979; Banerjee *et al.*, 1992) and 28.42 (Rao and Sreemannarayana, 1982).

5.1.1 SERUM PROGESTERONE CONCENTRATION

Serum samples evaluated at 10 days interval in subestrus and true anestrus revealed high (1.28 to 1.30) and low (0.80 to 0.85) ng/ml progesterone level, respectively. Similar trend was also observed for estradiol fluctuating at high and low levels around 6 to 22 pg/ml. These observations confirming the earlier findings of low concentration of progesterone and estrogen found up to ten months postpartum in Jaffarabadi buffaloes and the ovaries remained smooth and inactive (Dugwekar *et al.*, 2008). Similar results were reported by Narayan *et al.*, (1984), Jain and Pandey (1985) and Madan (1988) in Murrah buffaloes. In the present study, fluctuating high

serum progesterone LH, HL and HH levels were observed in subestruslactating buffaloes. On the other hand,consistently low serum progesterone concentration levels LL were observed from blood sampling collected at two TRUS examinations done at 10 days interval i.e., -10 days and on the day of initiationof treatment and associated with inactive ovaries in true anestrus lactating buffaloes (with no palpable structures).

5.1.2 OVARIAN FOLLICULAR ACTIVITY OF ANESTRUS LACTATING BUFFALOES

Monitoring of theovarian activityofanestrous buffaloes usingTRUS, revealed,presence of ovaries bearing small follicles 018/70 (25.7%), medium sized follicles 13/70 (18.5%), large follicles 3/70 (2.85%) and presence of corpus luteum was recorded in 34/70 (48.5%) lactating buffaloes. The buffaloes that had corpus luteum in one of theTRUSperformed at 10 days interval.

5.1.3 RELATIONSHIP OF OVARIAN FOLLICULAR ACTIVITY AND SERUM PROGESTERONE AND ESTRADIOL LEVEL IN ANESTROUS LACTATING BUFFALOES

In the present study, the anestrus buffaloes having small, mediumand large follicles at both the examinations (-10d and 0d) had low (LL) progesterone. The anestrus buffaloes that had HH, LH andHL progesterone concentration had either preovulatory follicle or corpus luteum. The buffaloes with LL and buffaloes with HH,LH and HL were confirmed as true anestrus and subestrus,respectively.

5.2 PATTERN OF INDUCED ESTRUS IN LACTATING ANESTRUS BUFFALOES

5.2.1 ESTRUS BEHAVIOR

The estrus detection was done at six hour interval after second PGF_{2α} injection in Group A (Double PG), after PGF_{2α} injection in Group B (Ovsynch group) afterPGF_{2α}injection on day 7 and implant removal on day 9 in group C (Norgestomet plus PMSG). All the buffaloes were observed for estrus signs in the morning and evening with the help of a teaser bull. Intense to intermediate estrus behaviour was

observed in more number of animals in the true anestrus animals treated with Norgestomet plus PMSG, compared to the other two groups, the estrous signs are pronounced and observable.

5.2.2 ESTRUS RESPONSE

In the present study, the estrus response after second PGF_{2α} injection in Double PG (Group A), after PGF_{2α} injection in Ovsynch (Group B) after PGF_{2α} injection, implant removal Crestar plus PMSG (Group C) and was 75, 75, 100 per cent respectively, whereas it was 36.36 per cent in control group (Group D). All the responded animals were detected in estrus after 24 hours and within four days (96 h) following the treatments in different groups.

5.3 TREATMENT OF SUBESTRUS BUFFALOES

5.3.1 Estrus response in subestrus buffaloes treated with double PG

The buffaloes included in this group were cyclic at the beginning of the program. Out of 16 subestrus buffaloes, 14/16 had MF, CL and 2/16 had LF, CL on day 0 i.e., on the day of first PGF_{2α} injection with a mean progesterone concentration of 1.30 ± 0.11 ng/ml. Following the first PGF_{2α} injection there was an abrupt decline in the size of the corpus luteum. Eight buffaloes had exhibited estrus within 72 hours but not inseminated. On day 11 at the time of second PGF_{2α} injection 12/16 had MF, CL 4/16 had large follicle. Following the second PGF_{2α} injection 12/16 subestrus buffaloes exhibited estrus. The progesterone concentration on the day of estrus was 0.72 ± 0.08 (0.3 to 1.20) ng/ml. The size of the ovulatory follicle varied from 9 to 13 (11.8 ± 1.02) mm.

The decline in the size (luteolysis) of the CL might have led to low progesterone and due to presence of large follicle the buffaloes might be induced to estrus (Starbuck *et al.*, 2006 and Dadarwalet *et al.*, 2009).

The 75 per cent estrus response observed in this study was lower than the earlier reports of Chauhan *et al.* (1981), Dhaliwalet *et al.* (1988), Chohan (1998),

Dadarwalet.al.(2009) and Kumar *et.al.* (2013) who reported 83.3, 85.5, 87.8,100 and 90 per cent estrus response.Srivastava(2005) and Pant and Singh (1991) reported 51 and 31 per cent estrus response, which is much lower than the present observation. The variation in the estrus response could be attributed to season, parity, type of feeding, managementand location.

5.3.2 Onset of estrus insubestrus buffaloes treated with double PG

The subestrus buffaloes treated with double PGF_{2α} injection had brought 12 buffaloes to estrus with in a mean time of 61.39.± 10.00(48 to 96) hours after second PGF_{2α} injection. These findings are in close agreement with the earlier observation of Srivastava*et.al.* (2005) and Pant and Singh (1991) who reported 69.3± 5.61 (48-72) and 67.25± 3.83 hours, respectively.

5.3.3 Intensity of estrus insubestrus buffaloes treated with double PG

The per cent of cows that showed intense, intermediate and weak estrus were 8.33 (1/12), 66.66(8/12) and 25.00 (3/12) in double PG group.In this treatment group only one animal had exhibited mounting behavior. Incidence of standing to be mounted type of estrus activity was poor. The important sign was mucus discharge, based on which estrus was detected. On per rectal examination uterine tone and cervical relaxation was observed in 8 buffaloes and mild tone with partial cervical relaxation was observed in 3 buffaloes. Similar observations were also reported by Srivastava (2005).

5.3.4 Ovulationrate insubestrus buffaloes treated with double PG

The ovulatory response in Double PG was 83.5 (10/12) per cent evidenced with presence of corpusluteum as detected by TRUS 10 days post A.I. Much lower and higher ovulation (66.6 vs83.5) and (92.85 vs.83.50) rate was reported byChauhan*et.al.* (1981) and Dhaliwalet.al.(1988), respectively.

5.3.5 Conception rate at induced estrus in subestrus buffaloes treated with Double PG

In buffaloes subjected to this protocol the first service conception rate in low breeding season was 58.33 (7/12) per cent. This was almost similar to the previous PGF_{2α} based strategies reported by Pant and Singh (1991) and Chohan (1998) who reported 54.8 and 53.1 per cent conception. Chauhan *et al.* (1981), Srivastava (2005) and Kumari *et al.* (2013) reported very low conception rate of 20.00, 40.00 and 44.44 per cent, respectively. The reason for variation in conception rate may be that the treatment was given during breeding, low breeding or summer season.

5.4 INFLUENCE OF SERUM PROGESTERONE CONCENTRATION ON CONCEPTION RATE

The influence of Serum progesterone concentration on the day 0 and day 11 and day 10 postAI on conception rate in double PG treatment was studied. The buffaloes that had Serum progesterone concentration of ≥ 1 ng/ml on the day of second PGF_{2α} associated with ovulation and conception.

5.5 TREATMENT OF TRUE ANESTROUS BUFFALOES

5.5.1 TRUE ANESTROUS BUFFALOES TREATED WITH OVSYNCH

5.5.1.1 Estrus response in true anestrous buffaloes treated with Ovsynch

In the present study, the estrus response after PGF_{2α} injection on day 7 in Ovsynch (Group B) was 75 (12/16) per cent. In Ovsynch the responded animals had a follicle greater than 10 (9 to 14) (12.1 \pm 2.01) mm and tonic uterus. Mucus discharges and estrus behavior were observed on rare occasion. In the present study, none of the animals exhibited visible estrus during the treatment period. However, various authors reported the incidence of premature estrus to be 3 (Burke *et al.*, 1996), 7 (Geary *et al.*, 2001) while using Ovsynch protocol.

The estrus response with Ovsynch (Group A) treatment in anestrous buffaloes of the present study (75%) was in total agreement with Atanasov *et al.* (2011). On the

contrary, the estrus response reported by Neglia *et al.* (2003), Malik *et al.* (2010) and Atanasov *et al.* (2011) was higher (75.00 vs. 88.00 and 87.50%) than the present observation. However, Ravi Kumar *et al.* (2005) and Azawi *et al.* (2012) reported much lower (41.66 and 35.00 %) estrus induction in Murrah and Iraqi buffaloes respectively. The buffaloes which responded to treatment in this group had a palpable follicle (12.1 ± 2.01), tonic uterus, cervical dilatation and other signs like cervical mucus discharge.

5.5.1.2 Onset of estrus in true anestrus buffaloes treated with Ovsynch

The true anestrus buffaloes treated with Ovsynch had brought 12 buffaloes to estrus within a mean time of 52.00 ± 1.70 (48 to 60) hours after $\text{PGF}_{2\alpha}$ injection on day 7. These findings are in close agreement with the earlier observation of Ravi Kumar *et al.* (2005), Malik *et al.* (2011) and Jabeen *et al.* (2012) who reported 48.80 ± 7.74 , 48.00 and 48.00 hours, respectively.

5.5.1.3 Intensity of estrus in true anestrus buffaloes treated with Ovsynch

True anestrus lactating buffaloes treated with Ovsynch had shown intermediate and weak estrus. No buffalo had shown intense estrus. The per cent of buffalo cows with intermediate and weak estrus were 50.0 (6/12) and 50.0 (6/12) in Ovsynch group. Tonic uterus and mucus discharge was observed in all the 12 estrus responded buffaloes. Estrus behavior like mounting on other animals or standing to be mounted was not observed in these animals. The behavior of buffaloes towards teaser bull was less intense in the present study. The understanding of physiological mechanisms behind the absence of intense behavioral estrus preceding ovulation remains an issue after estrus induction with $\text{PGF}_{2\alpha}$ in buffaloes (Brito *et al.*, 2002).

5.5.1.4 Ovulation rate in true anestrus buffaloes treated with Ovsynch

The ovulatory response in Ovsynch was 91.66 (11/12) per cent evidenced with presence of corpus luteum as detected by TRUS 10 days post A.I. The ovulation rate (91.66 %) recorded for the Ovsynch group was higher than the ovulation rate reported by several authors like Baruselli *et al.* (2003), Irikura *et al.* (2003), Presicce *et al.* (2005), Ravi kumar *et al.* (2005), Ali *et al.* (2008), Malik *et al.* (2010), Malik *et al.* (2011), and Derar *et al.* (2012) who recorded 60.60%, 80.00%, 81.81%, 82.00%, 83.30%, 85.70%, 87.50%, 88.00% respectively. Ali and Fahmy (2007) and Ghuman *et al.* (2009) recorded 100% ovulation rate in Ovsynch protocol. Yoto *et al.* (2012) recorded less (91.66 vs 55.60%) ovulation rate.

5.5.1.5 Conception rate in true anestrus buffaloes treated with Ovsynch

The conception rate at induced estrus for Ovsynch group recorded in the present study is 66.66 (8/12), is much higher than the conception rates recorded by Ravikumar *et al.* (2005), Ghuman *et al.* (2009), Malik *et al.* (2010) Malik *et al.* (2011) and Ghuman *et al.* (2012) who recorded 33.33 per cent, 18.00 per cent, 12.50 per cent, 20.00 per cent and 44.40 per cent respectively.

5.5.1.6 Ovarian activity

The ovarian activity in true anestrus buffaloes treated with Ovsynch was classified as SF, MF, LF, PF, CL; on day 0, day 7, day 9 and on estrus day. Out of 16 true anestrus buffaloes, 5/16 had SF, 11/16 had SF, MF on day 0, 12/16 had MF, LF on day 7, 13/16 had LF, PF on the day 9 and 15/16 had LF, PF on estrus day.

5.5.2 TRUE ANESTROUS BUFFALOES TREATED WITH NORGESTOMET AND PMSG

5.5.2.1 Estrus response in true anestrus buffaloes treated with Norgestomet plus PMSG

The administration of norgestomet (Crestar ear implant) along with PMSG induced estrus in all (16/16) the buffaloes with 100 per cent response and this observation was in total agreement with earlier reports (Rao *et al.*, 1982; Singh *et al.*,

1988; and Nayaket *al.*, 2009).The present estrus response rate was higher than the earlier reports of Agarwalet.*al.* (2001), Kumar *et.al.*(2010) and Mahesh *et.al.*(2011) who reported 71.42, 86.40 and 70.00 per cent estrus response, respectively.

5.5.2 .2 Onset of estrus in true anestrous buffaloes treated with Norgestomet plus PMSG

The 57.17 ± 2.09 (48 to 72) hours time interval to onset of estrus observed in this study was almost close to the earlier finding of 2.75 days and 65.14 ± 11.39 hours by Nayaket.*al.* (2009) and Caesar (2011), respectively.

5.5.2 .3 Intensity of estrus in true anestrous buffaloes treated with Norgestomet plus PMSG

The true anestrous lactating buffaloes in this group had exhibited 37.5 (6/16) 50.0 (8/16) and 12.5 (2/16) per cent intense, intermediate and weak estrus. The present finding of 37.5 percent intense estrus is in agreement with the findings of Chede(1990)and Nayaket.*al.*(2009) who reported 38.70 and 37.50 per cent, respectively. Kathiresan*et.al.*(1995) reported this as 28.50 per cent.

5.5.2.4 Ovulation rate in true anestrous buffaloes treated with Norgestomet plus PMSG

The ovulatory response 87.5 (14/16) per cent of low breeding season is in total agreement with Presicce*et.al.*(2005) who reported 88.2 per cent ovulation rate during non breeding season.

5.5.2.5 Conception rate in true anestrous buffaloes treated with Norgestomet plus PMSG

The 62.5 (10/16) per cent conception during low breeding season of the this study is lower than the earlier reports of Presicce*et.al.*(2005), Naiket.*al.*(2009).Caesar (2011) and Mahesh *et.al.*(2011) who reported 70.50, 75.00, 85.70 and 70.00 per cent respectively, and higher than the findings of Rao*et.al.*(1982);

Singhet *et al.* (1988) and Kumar *et al.* (2010) who reported 36.00, 50.00, 33.33 per cent conception, respectively.

5.6 COMPARISON OF REPRODUCTIVE PARAMETERS AMONG THE TREATMENT GROUPS.

5.6.1 ESTRUS RESPONSE

In subestrus buffaloes treated with double PG, 8 buffaloes exhibited estrus after first PG injection during the treatment period, which could be due to presence of large and preovulatory follicles and luteolysis of corpus luteum since the lactating buffaloes in this group were cyclic. None of the true anestrous lactating buffaloes treated with Ovsynch and Norgestomet plus PMSG exhibited premature estrus during the treatment period. The norgestomet (Group C) in the present study might have prevented the occurrence of premature estrus. The reason for non exhibition of estrus during the treatment period in the present study in Ovsynch group was mainly due to non availability of ovulatory follicles at the time of first GnRH as the buffaloes in this group were true anestrous consisting of inactive ovaries with small and medium sized follicles.

The reasons for higher estrus response in Norgestomet plus PMSG (group C) than Ovsynch group in the treatment of true anestrous buffaloes could be due to the fact that the Crestar injection containing norgestomet and estradiol valerate would have prevented ovulation, CL formation and at the same time induced luteal regression if CL is present. The Crestar ear implant would have created artificial luteal phase in buffaloes. After removal of the implant, the estrus response could be due to inhibiting block on gonadotropins was removed (Ravi Kumar *et al.*, 2005). Added to this the PGF_{2α} injection towards the end of the treatment would have induced luteolysis in the responded buffaloes. The non-response in some buffaloes could be attributed to low levels of P₄ concentrations and absence of a dominant follicle towards the end of the treatment period (De-Rensis *et al.*, 2005).

5.6.2 ESTRUS DURATION

The mean estrus duration of synchronized estrus was 22.08 ± 0.96 (19 to 32 hours), 16.83 ± 0.69 (14 to 22 hours), 24.33 ± 1.33 (18 to 34 hours) and 21.67 ± 3.9 (18 to 30 hours) in Double PG (Group A), Ovsynch (Group B), Crestar plus PMSG (Group C) and control (Group D) groups. In Ovsynch (Group B) group the mean estrus duration was 16.83 ± 0.69 (14 to 22) h and there is significant difference between treatment group and control. Kadu and Chede *et al.* (1992) reported similar observations as 34.06 ± 1.86 h duration in postpartum anoestrus buffaloes. The mean estrus duration reported in the treatment groups A and C is shorter than the earlier reports of Nayak *et al.* (2009) who reported > 48 h. The duration of estrus in postpartum anoestrus buffaloes treated with norgestomet plus PMSG has shown a practical advantage, because it has induced intense estrus effectively and allowed longer period to detect estrus and insemination buffaloes.

5.6.3 INTENSITY OF ESTRUS

Most buffaloes in the present study showed intermediate to weak estrus signs in Double PG (Group A) and Ovsynch (Group B) groups, on the contrary the Norgestomet plus PMSG showed intense to intermediate estrus in more (14/16) number of animals..

In the present study, intensity was classified as intense, normal and weak. The intense manifestation was highest in the Crestar plus PMSG group with 50.0 (8/16) per cent followed by intermediate and weak estrus in 37.5 and 12.5 (6/12 and 2/12) respectively. In Ovsynch group, intermediate and weak estrus was 50.0 (6/12) and 50.0 (6/12). None of the buffaloes exhibited intense estrus in this group. The subestrus buffaloes treated with Double PG exhibited 66.66 and 25.00 (8/12 and 3/12) per cent intermediate and weak estrus, respectively. Only one buffalo showed intense estrus. Numerically it is evident that progesterone based estrus induction treatments i.e., Crestar plus PMSG have shown intense estrus signs which include behavioral signs like

bellowing, excitement and standing to be mounted with mucus discharge from vulva were exhibited from 24 to 48h after ear implant (norgestomet) removal. High per cent of intense estrus observed with norgestomet in group C is in agreement with Narasimha Rao and Suryapakasham (1991).

The higher estrus response and intensity of estrus in Crestar plus PMSG group than the other two estrus induction treatments could be due to the fact that development of follicle to more than 6mm diameter needs gonadotropins. Maturation of follicle, development of LH receptors in the granulosa cells, production of estradiol from the follicles are FSH dependent events (Webb *et al.*, 1994; and McDougal *et al.*, 1995). As PMSG possesses both FSH and LH like biological action with FSH like action being dominant, the better estrus response and intensity was resulted in Crestar plus PMSG group.

5.6.4 DEGREE OF SYNCHRONY

Peak estrus synchrony with highest number of animals exhibited estrus between 48 to 72h with 7/12 in group A, 8/12 in group B and 10/16 in group C.

5.7 FERTILITY RESPONSE

5.7.1 OVULATION RATE

Ovulation rate response of 83.50 (10/12), 91.66 (11/12), 87.50 (14/16) Double PG (Group A) and Ovsynch (Group B) and Norgestomet plus PMSG.

5.7.2 CONCEPTION RATE AT INDUCED ESTRUS

Conception rate at induced estrus was calculated as percentage of animals that were conceived to insemination done at detected/ observed estrus in each group. The conception rate at induced estrus in the present study for treatment groups involving Double PG in the treatment of subestrus and Ovsynch and Norgestomet plus PMSG in the treatment of true anestrous buffaloes were 58.33 (7/12), 66.67 (8/12) and 62.50 (10/12) per cent, respectively.

5.8 SERUM HORMONAL PROFILE

5.8.1 Ovarian status assessment by serum progesterone concentration in induced ovulation and its influence on first service conception rate

The (16) buffaloes in this group were classified as buffaloes with high (H), low (L) progesterone into different combinations based on the serum progesterone levels. Viz., HH, HL, LH and LL. The HH and LH indicating high progesterone and HL low progesterone on the day of second PG.

In the present study, 8/16, 4/16 and 4/16 buffaloes included in group A (Double PG), had HH, LH and HL progesterone. The buffaloes that had HH had corpus luteum on both the occasions and HL, LH indicated presence of CL on day 0 and on the day 11 before second PG injection, as detected by TRUS. In the present study, in group A the estrus induction rate and conception rate at induced (first) estrus was 12/16 (75%) and 7/12 (58.33%) lactating buffaloes. The serum progesterone (0.72 ± 0.08) ng/ml level on the day of induced estrus was low and correlated with presence of preovulatory follicle. The buffaloes that had 1 ng/ml progesterone had failed to conceive.

In the present study 0/16, 2/16, 9/16 and 3/16; 1/16, 0/16, 13/16 and 2/16 true anestrus buffaloes included in group B and C had HHH, HHL, HLL, LLL, LHH and LHL and LLH levels of progesterone in each of the serum samples collected on d 0, d 7 and d 9 (second GnRH in group B and Crestar removal in group C) respectively. The incidence of estrus and first service conception rate of true anoestrus lactating buffaloes group B (Ovsynch) and C (Crestar plus PMSG) was 12/16, 16/16 and 50.00, 62.50 per cent respectively.

5.8.2 SERUM ESTRADIOL

In the present study, in true anoestrus buffaloes serum estradiol levels were low on day 0 in majority of the animals and elevated towards the end of the treatment in

a sustained manner. The serum estradiol levels were significantly high on the day of induced estrus ($P < 0.05$).

5.8.3 Influence of Serum progesterone on estrus induction and conception

The subestrus buffaloes which had high Serum progesterone concentration at the beginning of the treatment (day 0) and on day 11 at the time of second PG induced to estrus (12/16) and conceived (7/12) at first service.

The true anestrous buffaloes which have shown elevated progesterone, LLH (8/16), LHH (4/16) in Ovsynch (group B) and LLH (9/16), LHH (7/16) in group C (Crestar plus PMSG) 12/16, 16/16 buffaloes exhibited estrus and 8/12, 10/16 conceived at first (induced) estrus.

5.8.4 OVARIAN ACTIVITY IN TREATMENT GROUPS

The ovarian activity in subestrus buffaloes treated with double PG (group A) was classified as SF, MF, LF, PF, CL; on day 0, day 11 and on estrus day. Out of 16 subestrus buffaloes, 14/16 had MF, CL on day 0, 12/16 had LF, CL on day 11 and 12/16 had PF on the day of estrus day.

The ovarian activity in true anestrous buffaloes treated with Ovsynch was classified as SF, MF, LF, PF, CL; on day 0, day 7, day 9 and on estrus day. Out of 16 true anestrous buffaloes, 5/16 had SF, 11/16 had SF, MF on day 0, 10/16 had MF, LF on day 7, 13/16 had LF, PF on the day 9 and 15/16 had LF, PF on estrus day.

The ovarian activity in true anestrous buffaloes treated with Crestar plus PMSG (group C) was classified as SF, MF, LF, PF, CL; on day 0, day 7, day 9 and on estrus day. Out of 16 True anestrous buffaloes, 5/16 had SF, 10/16 SF and 6/16 had MF on day 0, 12/16 had MF, LF on day 7, 13/16 had MF, LF on the day 9 and 15/16 had LF, PF on estrus day.

5.9 Overall conception rate

In the present study overall conception rate among the true anestrous buffaloes treated was higher 13/16 (81.25%) in Crestar plus PMSG group followed by 75 per cent each in Double PG (Group A) and Ovsynch (Group B).The conception rate in control group was only 62.5 (5/8).

The overall conception rate between Double PG (Group A) and control revealed a significant ($P < 0.05$) difference. This could be attributed to follicles which ovulated subsequent to first PG induced luteolysis could be at different developmental stages on day 0. On day 0 in this group of animals the size of the follicles were SF,MF and LF. Due to luteolysis the decline in progesterone these follicles might have developed in a synchronized manner to preovulatory stage and induced estrus after luteolysis with second PG.

In the true anestrous buffaloes, different response to treatment was observed in buffaloes with low progesterone concentration at the starting of treatment. Higher ovulation and pregnancy rates were recorded after progesterone treatment compared to the Ovsynch protocol. In the present study all the buffaloes are in anestrous with low progesterone concentrations. The reason for the poor pregnancy rates following Ovsynch treatment in buffaloes with low progesterone concentration could be due to anovulatory condition (Lopez - Gatiuset *al.*,2001, Wiltbanket *al.*, 2002). Theovulatory condition, probably due to low GnRH responsive follicles at the time of first GnRH injection of the Ovsynch protocol. It has been shown that ovulation of the follicles in response to the first GnRH injection is a prerequisite for the success of the Ovsynch protocol (Vasconceloset *al.*, 1999). The limited response to the treatment with GnRH and PGF_{2 α} in anestrous cows with Ovsynch protocol with a persistent follicle (Lopez - Gatiuset *al.*,2001).

In the Norgestomet plus PMSG treatment group estrus, ovulation, and conception rates were higher in anestrous buffaloes with low progesterone

concentration at the time of treatment onset. The combination of Norgestomet plus PMSG seems to be sufficient to facilitate follicular growth and maturation before ovulation after removing the implant leading to improved ovulation and pregnancy (Singh *et al.*, 1998; Agarwalet *et al.*, 2001).

Equine CG increased ovulation rate in buffalo subjected to TAI (Singh *et al.*, 1983). In buffalo, Murugavelet *et al.* (2009) reported a greater ovulation rate in controlled Intravaginal drug release (CIDR) with eCG (81.0%) compared with CIDR (47.4%) treatment in noncyclic buffalo cows. Anestrous cows have insufficient LH pulsatile release to support the final stages of ovarian follicular development and ovulation. The efficiency of eCG is related to its FSH- and LH-like activities, Murphy and Martinuk ,(1991) which ensure continuation of ovarian follicular growth in cows with compromised gonadotropins secretion. In this regard, Baruselliet *et al.*, 2003, confirmed that the effect of eCG was more pronounced with deeper anestrous in *Bosindicus* breeds. Thus, improved fertility after eCG supplementation observed in the present study might have been because of an increase in diameter of the ovulatory follicle and the percentage of cows that ovulated.

As per the progesterone concentrations recorded in the Ovsynch treatment group the poor conception rates in the Ovsynch protocol in the treatment of true anestrous buffaloes could be due to fact that anestrous buffaloes would have responded to first GnRH injection, but the PGF_{2α} induced incomplete luteolysis might have resulted in a persistent follicle (anovulatory) (Twagiramunguet *et al.*, 1994) that would have ovulated following administration of a second dose of GnRH, 24 to 48 h after PGF_{2α}, due to LH release caused by GnRH (Wiltbanket *et al.*, 1996). The possible retention of Graafian follicle for an extended period of time might have lead to ageing of oocyte. In the two conceived animals the progesterone concentrations were less on day 9 which could be due to complete luteolysis and bringing the ovulation of a fertilizable oocyte.

The other possible explanation for a poor estrus expression, ovulation and conception in Ovsynch protocol could be due to lack of progesterone concentration subsequent to first GnRH injection and on day 7 prior to PGF_{2α} injection.

The results of present study with inseminations at observed estrus appears as an effective way of managing reproduction in subestrus buffaloes with luteal activity using double injection prostaglandins and true anestrous buffaloes either non cyclic or in the follicular phase of the estrous cycle with Ovsynch and Norgestomet plus PMSG as we could record acceptable ovulation and conception rates. The treatment of true anestrous buffaloes with Norgestomet plus 500 I.U of PMSG appears to have some beneficial effects with 100 per cent estrus response, intense estrus in an observable manner, 87.5 per cent ovulation rate and higher overall (81.25%) conception rate.

Future studies to be conducted

Detailed study of follicular dynamics by Transrectalultrasonography in trueanestrous buffaloes before and during treatments using progesterone alone and progesterone with PMSG (eCG) or HCG are needed for dealing with postpartum lactating anestrous buffaloes as a therapeutic measure.

SUMMARY

In the present investigation, critical differential diagnosis of the buffaloes that failed to exhibit estrus beyond 180 to 270 days postpartum using trans rectal ultrasonography revealed incidence of 51.4 (36/70) and 48.6 (34/70) per cent of true anestrus and subestrus, respectively.

Treatment of subestrus and true anestrus lactating graded Murrah buffaloes (*Bubalus bubalis*) using estrus and ovulation hormonal protocols, Double PGF_{2α} (Group A), Ovsynch (Group B) and norgestomet plus PMSG (Group C) and their influence on fertility during low breeding season was studied.

Following the treatment, with Double PGF_{2α} (Group A) 8 buffaloes exhibited estrus to the first PGF_{2α} injection between day 0 to 11 of the treatment protocol. In the other two treatments with Ovsynch (Group B) and norgestomet plus PMSG (Group C) none of the true anestrus buffaloes exhibited estrus between day 0 to 9. The estrus detection rate was 75 (12/16), 75 (12/16) and 100 (16/16) per cent in Double PGF_{2α} – subestrus, Ovsynch and norgestomet plus PMSG- true anestrus groups.

There was no significant difference in mean time to onset of estrus among the three treatments. Most buffaloes in the present study showed intermediate to weak estrus signs in Double PG (Group A) and Ovsynch (Group B) groups, on the contrary, the Norgestomet plus PMSG showed intense to intermediate estrus in more (14/16) number of lactating anestrus buffaloes.

Peak estrus synchrony with highest number of animals exhibited estrus between 48 to 72h with 7/12 in group A, 8/12 in group B and 10/16 in group C. The artificial insemination submission rate was 75 (12/16), 75 (12/16) and 100 (16/16) per cent in Double PGF_{2α} -subestrus, Ovsynch, and norgestomet plus PMSG, respectively.

Ovulation response of 83.5 (10/12), 91.66 (11/12), 87.5 (14/16) per cent was observed in Double PG (Group A) and Ovsynch (Group B) and Norgestomet plus PMSG, respectively.

The conception rate at induced estrus in the present study for treatment groups involving Double PG in the treatment of subestrus and Ovsynch and Norgestomet plus PMSG in the treatment of true anestrus buffaloes were 58.33 (7/12), 66.67 (8/12) and 62.5 (10/12) per cent, respectively. However, the overall conception rate was 75 (9/12), 75 (9/12) and 81.25 (13/16) per cent respectively in the above groups.

Serum samples collected on d 0, d 11 in treatment groups involving Double PG in the treatment of subestrus group A, 12 /16 and 4/16 has shown, HH and LH indicating high progesterone and low progesterone on the day of second PG. The buffaloes that had HH serum progesterone had corpus luteum on both the occasions and HL, LH indicated presence of CL on day 0 and on the day 11 before second PG injection, as detected by TRUS.

In the present study, 0/16, 2/16, 9/16 and 3/16 true anestrus buffaloes included in group B (Ovsynch) had small, medium and preovulatory follicles with HH, HL, LH, and LH levels of progesterone in each of the serum samples collected on d 0 and d 7 respectively during the treatment and associated with low progesterone concentration and presence of preovulatory follicles on the day of estrus. In the treatment group C (Crestar plus PMSG) 1/16, 0/16, 13/16 and 2/16 had small, medium, large and preovulatory follicles with LLH, LLL, LHL and LHH levels of progesterone on d 0, 7 and d 9 and in all the estrus responded buffaloes the presence of preovulatory follicles associated with low progesterone.

In the present study, overall conception rate among the true anestrus buffaloes treated was higher 13/16 (81.25%) in Crestar plus PMSG group followed by 75 per cent

each in Double PG (Group A) and Ovsynch (Group B). The conception rate in control group was only 62.5 (5/8).

The overall conception rate between Double PG (Group A) and control revealed a significant ($P < 0.05$) difference. This could be attributed to follicles which ovulated subsequent to first PG induced luteolysis could be at different developmental stages on day 0. Due to luteolysis the decline in progesterone, these follicles might have developed in a synchronized manner to preovulatory stage and induced estrus after luteolysis with second PG.

In the true anestrus buffaloes, different response to treatment was observed in buffaloes with low progesterone concentration at the starting of treatment. Higher ovulation and pregnancy rates were recorded after progesterone treatment compared to the Ovsynch protocol.

In the Norgestomet plus PMSG treatment group estrus, ovulation, and conception rates were higher in anestrus buffaloes with low progesterone concentration at the time of treatment onset. The combination of Norgestomet plus PMSG seems to be sufficient to facilitate follicular growth and maturation before ovulation after removing the implant leading to improved ovulation and pregnancy.

The results of present study with inseminations at observed estrus appears as an effective way of managing reproduction in subestrus buffaloes with luteal activity using double injection prostaglandins and true anestrus buffaloes either non cyclic or in the follicular phase of the estrous cycle with Ovsynch and Norgestomet plus PMSG as we could record acceptable ovulation and conception rates. The treatment of true anestrus buffaloes with Norgestomet plus 500 I.U of PMSG appears to have some beneficial effects with 100 per cent estrus response, intense estrus in an observable manner, 87.5 per cent ovulation rate and higher overall (81.25%) conception.

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