



## Fertility Response using Timed Insemination Protocols in Sub-oestrus Buffaloes

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### ABSTRACT

Fertility response to fixed time artificial insemination protocol was studied in 45 sub-oestrus buffaloes divided randomly into three groups (n=15). Animals of group-I were administered intravaginal progesterone implant for 7 days along with GnRH (day 0) followed by PGF<sub>2</sub>α (day 7). Animals of group-II were administered GnRH (day 0), followed by PGF<sub>2</sub>α (day 7) and a second GnRH (day 9). Animals of group-III were treated similar to group-II followed by long acting biphasic Insulin on day 9, 10 and 11. Timed artificial insemination was performed on 10<sup>th</sup> and 11<sup>th</sup> day at an interval of 24 hours in all the groups. Based on the intensity of the oestrus signs observed during fixed time artificial insemination the oestrus synchronization was graded as excellent, good, fair, poor and nil. The results revealed better grades of oestrus synchronization in insulin modified Ov-synch protocol (GIII) as compared to the original Ov-synch (GII) and progesterone implant (GI) group. The higher conception rate was recorded in insulin modified Ov-synch (73.33%), followed by progesterone implant (60.00%) and Ov-synch protocol (46.66%).

**Keywords:** Sub-oestrus, Buffalo, Ov-synch, Progesterone implant, Timed AI

Buffalo is considered as the animal of poor reproductive efficiency due to several features including delayed puberty and sexual maturity, sub-oestrus, anoestrus and seasonality in breeding. Ovarian disorders are the most important causes of infertility characterized by cessation of sexual cycles and psychic manifestation of oestrus in dairy animals. Anoestrus is a functional disorder of ovaries causing lowered fertility in dairy animals especially in buffaloes and responsible for tremendous economic losses to the farmers by decreasing calf crop and milk production. The term sub-oestrus is used for the unobserved and silent oestrus responsible for prolonged calving interval and culling at breeding age in buffaloes. It is erroneously considered as anoestrus in field conditions. Incidence of sub-oestrus in dairy buffaloes has been reported 21.05 to 35.13 per cent under organized farms and 37.64 per cent in unorganized rearing systems (Sachan, 2013 and Singh, 2013).

The oestrus synchronization and fixed time artificial

insemination (AI) was introduced as an alternative to improve reproductive efficiency in buffalo herds. Various oestrus synchronization protocols using progesterone, prostaglandins (PGF<sub>2</sub>α) and gonadotrophic releasing hormone (GnRH) in different combinations have been tried to overcome oestrus detection problems and facilitate artificial insemination (AI) program (Singh *et al.*, 2000; Borghese, 2005). However, each and every synchronization protocol has its own merits and demerits.

Recently, Singh (2013) observed better fertility response in terms of oestrus induction and conception by natural service at induced oestrus in Insulin modified Ov-synch protocols as compared to the original Ov-synch protocol. The satisfactory synchronization and conception rate in postpartum as well as anoestrus buffaloes following use of insulin modified Ov-synch protocols were also recorded (Singh, 2014; Gupta *et al.*, 2015). Insulin has also been found effective for therapeutic management of anoestrus in buffaloes (Gupta *et al.*, 2010).



Insulin is a metabolic hormone enhances growth and proliferation of granulosa, theca and luteal cells present in the ovary (Spicer *et al.*, 1993; Stewart *et al.*, 1995). This stimulate folliculogenesis either acting through specific insulin receptor and IGF-1 or both type of the receptors (Adashi *et al.*, 1985; Poretsky *et al.*, 1985 and Kahn *et al.*, 1989). Insulin also induces LH pulse secretion and thus maturation of follicles (Tanaka *et al.*, 2000). The beneficial effects of insulin on resumption of ovarian cyclicity and fertility may be due to its action on the folliculogenesis and steroidogenesis (Gong *et al.*, 1994; Stewart *et al.*, 1995). As insulin is a non-steroidal metabolic hormone, cost effective and easily available thus can be utilized in modified Ov-synch based protocol to improve fertility response in sub-oestrus buffaloes.

## MATERIALS AND METHODS

### Animals

Postpartum dairy buffaloes maintained at Livestock farm of Veterinary College, and organized buffalo herds of Jabalpur (M.P.) were used for this study. Selection of sub-oestrus buffalo was made by history of anoestrus and gynaecological examination of genitalia twice at 10 days interval. Animal having clinically functional ovaries (palpable follicles or corpus luteum) were tentatively diagnosed as sub-oestrus and selected for the experiment. Sub-oestrus buffaloes were further confirmed by ex-foliate vaginal cytology (change in pattern of predominant cells) and serum progesterone assay (serum p<sub>4</sub> concentration less than 1 ng/ml during follicular phase and above 1ng/ml during luteal phase) at an interval of 10 days.

### Chemicals

Gonadotrophin Releasing Hormone (GnRH) analog containing Buserelin acetate 0.0042 mg equivalent to 0.004 mg Buserelin per ml. Prostaglandin F<sub>2</sub> alpha (PGF<sub>2</sub>α) analog containing 263 µg Cloprostenol Sodium B.P. Vet equivalent to Cloprostenol 250 µg per ml. Insulin (long acting biphasic Human Insulin) containing 100 IU long acting biphasic Human Insulin IP per ml. Progesterone implant contain progesterone IP 586 mg. The quantitative determination of progesterone concentration in serum was done using 96-wells Progesterone Enzyme Linked

Immuno sorbent assay (ELISA) kit, manufactured by Biochem diagnostics, Canada and Exfoliative vaginal cytology using Papanicolaous Stain Kit (RAPID-PAP, Biolab Diagnostics, Pvt. Ltd).

### Semen

Frozen Semen of murreh buffalo in French mini straws supplied by Government semen collection center, Bhadbhada, Bhopal, Madhya Pradesh was used for artificial insemination.

### Experimental design

The selected sub-oestrus buffaloes were divided randomly into three groups, each comprising 15 animals (n=15). Animals of group-I were administered intravaginal P<sub>4</sub> implant for 7 days along with GnRH analog Buserelin acetate (20 µg) intramuscularly on day 0 followed by cloprostenol (500µg) on day 7. Animals of group-II were administered Buserelin acetate (20 mg) intramuscularly at day 0, followed by Cloprostenol (500 mg) intramuscularly on day 7 and same dose of second GnRH on day 9. Animals of group-III were administered Buserelin acetate (20mg) intramuscularly at day 0 and Cloprostenol (500mg) intramuscularly at day 7 and second dose of GnRH on day 9 followed by long acting biphasic Insulin (@ 0.25 IU/Kg b.wt.) subcutaneously on day 9, 10 and 11. Timed artificial insemination was performed on day 10<sup>th</sup> (12-14 hrs. after GnRH administration in group-II and III) and 11<sup>th</sup> day at an interval of 24 hours in all the groups. Fertility response was studied in terms of oestrus synchronization and conception rate. Oestrus synchronization pattern was graded as excellent, good, fair, poor and nil based on the intensity of the oestrus signs observed during fixed time artificial insemination as per the study of Gupta *et al.* (2015).

## RESULTS AND DISCUSSION

Fertility response to fixed time artificial insemination protocol in sub-oestrus buffaloes was assessed in terms of oestrus synchronization and conception rate. The results of the study revealed that the insulin modified Ov-synch protocol (GIII) has better oestrus synchronization as compared to the original Ov-synch (GII) and progesterone implant(GI) group (Table 1). Better oestrus synchronization

in insulin modified Ov-synch protocol in the present study is comparable to the study of Singh (2014) who reported 100.00% synchronization and 83.32% conception in post partum buffaloes. Gupta *et al.* (2015) also reported 80.00% synchronization in post partum anoestrus buffaloes using insulin modified Ov-synch protocol.

**Table 1:** Grading of oestrus synchronization and conception rate in postpartum sub-oestrus buffaloes

Grades of oestrus synchronization	Group I (n=15)	Group II (n=15)	Group III (n=15)
Excellent	3(20.00%)	4 (26.66%)	6 (40.00%)
Good	6 (40.00%)	6 (40.00%)	6 (40.00%)
Fair	2 (13.33%)	3 (20.00%)	2 (13.33%)
Poor	3(20.00%)	1(6.66%)	1 (6.66%)
Nil	1(6.66%)	1(6.66)	0 (0.00%)
Conception rate (%)	60.00	46.66	73.33

In this study, the higher conception rate was recorded in insulin modified Ov-synch (73.33%, GIII) followed by progesterone implant (60.00%, GI) and Ov-synch (46.66%, GII) ( $p>0.05$ ). Conception rate obtained in the present study using conventional Ov-synch was comparable with the results of Berber *et al.* (2002), Renesis *et al.* (2005), Baruselli *et al.* (2007) and Singh (2014). However, lower conception rate was reported by Paul and Prakash (2005) who suggested that unsatisfactory low conception rate in buffaloes may be due to early ovulation and sub-functional corpus luteum. Renesis *et al.* (2005) reported that presence of large follicles at the beginning of Ov-synch protocol is a determining factor for successful synchronization of ovulation and high conception in buffaloes. The literature is lacking regarding the use of insulin modified Ov-synch protocol for fixed time artificial insemination in sub-oestrus buffaloes as in the present study. By use of insulin modified Ov-synch protocol Singh (2014) found highest conception in postpartum buffaloes (66.66%) where second dose of GnRH was completely replaced by insulin on day 8, 9 and 10 followed by Ov-synch protocol using half dose of GnRH plus additional administration of insulin on day 8, 9 and 10 (58.33%). In another study, Singh (2013) found better conception (75.00-88.89%) using insulin modified Ov-synch protocols in postpartum anoestrus buffaloes. The comparatively poor result in this study may be due to breeding of buffaloes by fixed time AI using frozen semen without detection of oestrus

as compared to natural service in previous studies. The insulin modified Ovsynch protocols were also found effective in postpartum anoestrus buffaloes where upto 80.00% synchronization and 40.00% conception was recorded (Gupta *et al.*, 2015).

The beneficial effects of insulin may be due to its action on the folliculogenesis and steroidogenesis (Gong *et al.*, 1994; Stewart *et al.*, 1995). Insulin enhances growth and proliferation of granulosa, theca and luteal cells present in the ovary (Spicer *et al.*, 1993; Stewart *et al.*, 1995). This enhances folliculogenesis either acting through specific insulin receptor and IGF-1 or both type of the receptors (Adashi *et al.*, 1985; Poretsky *et al.*, 1985 and Kahn *et al.*, 1989). Insulin also induces LH pulse secretion and thus maturation of follicles (Tanaka *et al.*, 2000) which may be the reason of better oestrus signs and ovulation in this study.

The result of conception in the present study using progesterone implant was also comparable to the study of Guman *et al.* (2014) and Singh *et al.* (2009) using CIDR and PRID based oestrus synchronization protocol in sub-oestrus and anoestrus buffaloes, respectively. These results support the hypothesis that priming of hypothalamic system with adequate concentration of blood progesterone during pre-conception period is required for optimum growth of ovulatory follicle and thus corpus luteum development as well as priming of uterine endometrium (Forman *et al.*, 1990; Mc Neil *et al.*, 2006). However, in the study of Buhecha *et al.* (2016) the conception was found lower (25%) using progesterone implant only in anoestrus buffaloes. The beneficial effects of insulin alone and in various combinations using GnRH and PMSG in anoestrus cattle and buffaloes were also reported by Shukla *et al.* (2005a, b), Ramoun *et al.* (2007), Gupta *et al.* (2010) and Kumar *et al.* (2013) which supports result of the present study.

## CONCLUSION

It may be concluded that administration of insulin in Ov-synch protocol found more effective than progesterone implant with GnRH in sub-oestrus buffaloes.

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