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Fertility response to modified progesterone based synchronization protocol in true anoestrus buffaloes (*Bubalus bubalis*)*

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ABSTRACT

Present study was conducted on 42 true anoestrus buffaloes to observe the efficacy of controlled internal drug release (CIDR) implant alone and in combination with pregnant mare serum gonadotropin (PMSG) and Estradiol valerate for induction of estrus and fertility response. All experimental animals were randomly divided into 6 groups of 7 animals each. All the animals were administered 20 ml liquid terramycin intra uterine except in G1 and G3. Animals of G1 and G2 were served as control where as in G3, G4, G5 and G6 implanted with CIDR. Animals of G5 were administered 1 mg estradiol and G6 with 500 IU PMSG on the day of implant withdrawal. The serum progesterone and estrogen profiles were also studied before treatment, at estrus and 10th day post estrus. On removal of implant all the animals of G5 and G6 exhibited estrus within 30.42±5.10 and 65.14±11.39 h with 57.1 and 85.7% conception rate, respectively. However, in the animals of G4 and G3 induction rate was 85.7 and 71.4% within 40.08±2.09 and 72.00±10.76 h of onset interval with 66.6% and 0.00% conception rate, respectively. None of the animals of G1 and G2 induced in estrus. Serum progesterone (P_4) and estradiol (E_2) profiles confirmed our findings of rectal palpation. Study indicates that addition of PMSG to a progesterone based estrus synchronization regimen substantially improves ovulation rate and fertility in non cyclic buffaloes.

Key words: Anoestrus, Buffalo, Estradiol, CIDR, PMSG, Progesterone

Buffalo is a difficult breeder because of its inherent susceptibility to environmental stress leading to anoestrus and sub estrus condition. These two conditions are responsible for prolonged inter calving period resulting in great economic losses to dairy industry. Clinical survey revealed higher incidences of anoestrus and inactive ovaries in buffaloes (55.5 and 19.4%) than in cows (43 and 17.2%, respectively) (Tanwar *et al*. 2003). Exogenous administration of progesterone exerts a negative feedback effect over the hypothalamus and pituitary which blocks the release of pituitary gonadotrophin. Upon withdrawal of progesterone, the block is removed and larger quantities of gonadotrophins are released which in turn ensures growth and maturation of ovarian follicle and thus onset of estrus. Therefore, in view of the above, this study was conducted to observe the effect of controlled internal drug release (CIDR) alone and in combination with pregnant mare serum gonadotropin (PMSG) and estradiol on fertility response in anoestrus buffaloes.

MATERIALS AND METHODS

Animals and experiment groups: Study was conducted on 42 post partum (120 days onwards) true anoestrus buffaloes belonging to organized private dairy farms and Livestock Farm, Adhartal, Jabalpur. The true anoestrus condition was confirmed by 2 rectal palpations at 11 days interval and serum progesterone assay. Animals having history of anoestrus, smooth ovaries in both the examinations and less than 1 ng/ml serum progesterone level were selected for the study. All the experimental animals were randomly divided equally (n=7) into 6 groups. After confirmation of true anoestrus, 20 ml of liquid terramycin was administered intra uterine in all the animals except in G1 and G3 to rule out any subclinical infection of the genital tract. The CIDR was implanted intra vaginally to the animals of G3, G4, G5 and G6 for 9 days. Animals of G5 and G6 were also

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administered with 1 mg estradiol and 500 IU of PMSG on implant withdrawal, respectively. The animals of G1 served as control for the animals of group G2 whereas, animals of G2 served as control for the animals of G3 to G6. The treatment regimen followed was as under:

Groups	Treatment
G1	Untreated control
G2	20 ml liquid terramycin only
G3	CIDR alone
G4	CIDR + 20 ml liquid terramycin
G5	CIDR + 20 ml liquid terramycin + intramuscular injection of 1 mg estradiol valerate on the day of CIDR application and on the day of its withdrawal
G6	CIDR+ 20 ml liquid terramycin followed by intramuscular injection of 500 IU PMSG on the day of CIDR withdrawal

Material used: CIDR contains 1.38 g progesterone in one insert, PMSG contains 1000 IU/vial, estradiol valerate contain 10 mg/ml, liquid terramycin I/U contains 50 mg oxytetracycline hydrochloride/ml.

Estrus was detected by parading a buffalo bull followed by observing behavioral symptoms and confirmed by rectal examination of genitalia, and serum estrogen and progesterone estimation. Animals showing estrus were bred with fertile bull. The time taken for onset of estrus following withdrawal of treatment and fertility at induced and subsequent estrus was recorded and analyzed. Confirmation of pregnancy by per rectal method was done on 60 days after breeding.

Serum progesterone and estrogen assay: The serum progesterone and estrogen concentrations were determined quantitatively before start of treatment, at estrus and day 10 post estrus using ELISA assay kits.

Statistical analysis: The data of serum progesterone and estrogen profile were statistically analyzed as per the statistical methods of Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Fertility response: All the animals of estradiol (G5) and PMSG treatment (G6) were completely responded to treatments for estrus induction (100%) followed by animals of G4 (85.7%) and G3 (71.4%). However, none of the animals were induced to estrus in both the control groups (G1 and G2). The shortest duration of estrus induction was observed in animals of G5 (30.42±5.11 hrs) followed by G4, G6 and G3 (40.08±2.9, 65.14±11.39 and 72.00±10.76 hrs, respectively). The best conception rate was observed in G6 (85.7%) followed by G4 (66.6%) and G5 (57.1%). However, none conceived in CIDR alone group. Our finding for 100% estrus induction with in shortest duration in animals of G5 (CIDR + Estradiol) was in accordance with the findings of Nikam *et al.* (2002) who also reported 100% estrus induction within 35.66±3.98 hrs using Crestar ear implant along with estradiol valerate on the day of implantation. Our findings were in accordance with the reports of Nayak *et al.* (2009) who also obtained 85.7% estrus induction with 71.42% conception in buffaloes using Crestar implant for 7–days and an intramuscular injection of 2 ml crestar solution (3 mg norgestomet and 5 mg estradiol valerate) on the day of implantation.

The shortest duration for the onset of estrus with 100% induction in CIDR + estradiol group might be due to the administration of estradiol valerate on the day of implantation and withdrawal of CIDR. The estradiol 17-β is necessary for the pulsatile LH secretion that is prerequisite for maturation and ovulation of follicle and expression of estrus. It also induces premature regression of corpus luteum and enhances response to progestagens (Jainudeen *et al*. 2000).

Our results of fertility response in G6 (CIDR+PMSG) are in accordance with the findings of Dabas and Bardhan (2006), who treated anoestrus buffaloes with PMSG, progesterone and hCG. All the buffaloes were induced to estrus (100%) within 72 to 120 h with 100% conception. Our findings regarding induction of estrus, duration for onset, and conception rate are very close to the finding of Nayak *et al*. (2009) who also reported 100% estrus induction, within 2.75±0.249 days with 75% conception using crestar implant for 7-days with 2 ml of crestar solution and an intramuscular injection of 500 I U PMSG on the day of implant in postpartum anoestrus buffaloes.

The better conception rate (85.7%) in animals of G6 in comparison to G4 and G5 might be due to the synergistic effect of implant withdrawal with intramuscular injection of PMSG which stimulate follicular development and ovulation (Murugavel *et al*. 2000). Our results indicated that the

Table 1. Treatment response for estrus induction and conception

Groups	Animals (n)	Induction rate (%)	Onset of estrus interval (h)	Animals bred	Conception rate at induced estrus (%)
G1	7	-	-	-	-
G2	7	-	-	-	-
G3	7	5 (71.4)	72.00±10.76	5	0 (0.0)
G4	7	6 (85.7)	40.08±2.94	6	4 (66.6)
G5	7	7 (100)	30.42±5.10	7	4 (57.1)
G6	7	7 (100)	65.14±11.39	7	6 (85.7)

Table 2. Serum progesterone profile

Treatment groups	Day before start of treatment (ng/ml)	At estrus (ng/ml)	Day 10 post estrus (ng/ml)
G1 (Untreated control)	0.57±0.14	-	-
G2 (liquid terramycin)	0.43±0.09	-	-
G3 (CIDR alone)	0.45±0.09 ^{a}	0.25±0.05^{a}	3.6±0.22 $^{A\ b}$
G4 (CIDR + liquid terramycin)	0.37±0.07 ^{a}	0.49±0.08^{a}	4.1±0.40 $^{AB\ b}$
G5 (CIDR + liquid terramycin + estradiol valerate)	0.46±0.08 ^{a}	0.30±0.04^{a}	4.7±0.45 $^{C\ b}$
G6 (CIDR+ liquid terramycin +PMSG)	0.43±0.08 ^{a}	0.24±0.03^{a}	4.2±1.6 $^{BC\ b}$

Mean value bearing different superscripts (a,b) in rows and (A, B,C) in columns differ significantly (P<0.05).

Table 3. Serum estradiol-17β profile

Treatment groups	Day before start of treatment (ng/ml)	At estrus (ng/ml)	Day 10 post estrus (ng/ml)
G1 (control)	13.62±0.63	-	-
G2 (liquid terramycin)	14.13±0.40	-	-
G3 (CIDR alone)	14.11±0.43^{a}	40.47±2.66 $^{A\ b}$	18.53±1.07 $^{A\ c}$
G4 (CIDR + liquid terramycin)	13.42±0.39^{a}	45.97±0.60$^{B\ b}$	14.32±0.29 $^{B\ ac}$
G5 (CIDR + liquid terramycin + estradiol valerate)	14.22±0.43^{a}	43.61±1.86$^{B\ b}$	15.43±1.09 $^{B\ ac}$
G6 (CIDR+ liquid terramycin +PMSG)	13.9±0.31^{a}	44.83±1.49$^{B\ b}$	15.68±0.83 $^{AB\ ac}$

Mean value bearing different superscript (a, b,c) in rows and (A,B) in columns differ significantly (P<0.05).

addition of PMSG to a progesterone based estrus synchronization regimen substantially improve ovulation rate and fertility in non cyclic buffaloes.

Serum progesterone and estrogen assay: Serum progesterone concentration before treatment was less than 1.0ng/ml (ranged 0.37±0.071 to 0.57±0.14 ng/ml) in all the animals which confirmed the state of anestrous (Table 2). Similar observations were also reported by Gupta (2008). The basal level of serum progesterone (0.24±0.03 to 0.49±0.081 ng/ml) at induced estrus, increased thereafter and reached to highest value (3.6±.0.22 ng/ml, G3), (4.1±0.40 ng/ml, G4), (4.7±0.45 ng/ml, G5) and (4.2±0.37 ng/ml, G6) on day 10th post estrus indicating presence of functional corpus luteum. Our results are in agreement with the findings of Jainudeen *et al.* (1983), Kumud (1999) and Gupta (2008).

Rao and Pandey (1982) reported the poor concentration of progesterone at estrus (0.14±0.05 ng/ml) and diestrus (2.05±1.16 ng/ml) during summer as compared to 0.49±0.06 and 3.11±0.20ng/ml during winter season, respectively. However, the progesterone concentration at day 10th post estrus in CIDR alone (G3) was significantly lower as compared to other groups (P<0.05) which may be the cause for failure of conception due to luteal insufficiency.

The serum estradiol-17β concentration ranged 13.42±0.39 to 14.2±0.43 pg/ml before treatment indicates true anoestrus condition. Nearly similar observations in anestrous buffaloes were also reported by Batra and Pandey (1982) and Gupta (2008).

The concentration of serum estradiol-17ß at induced estrus was highest and ranged between 40.47±2.66 to 45.97 ± 0.60 pg/ml in all the groups however, it was significantly low in CIDR alone group as compared to other treatments (P<0.05) (Table 3). The findings are in close agreement with reports of Dugwekar *et al.* (2008), who also recorded 40.20±19.68 pg/ml estradiol-17β at estrus in 6 Jafarabadi buffaloes. Similar observations were also reported by Gupta (2008) in buffaloes at estrus.

The concentration of serum estradiol-17β on day10 post estrus was significantly higher in CIDR alone as compared to other treatment groups (P<0.05). This may be the reason for no conception in G3 animals which were treated with CIDR alone. The present study indicated that addition of PMSG and estradiol to a progesterone based estrus synchronization regimen substantially improves ovulation rate and fertility in non cyclic buffaloes.

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