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Efficacy of Norgestomet ear implant for estrus induction on postpartum anestrus Murrah buffaloes *(bubalus bubalis)*

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ABSTRACT

The study was conducted on 15 lactating, suckled, anestrus Murrah buffaloes to evaluate the efficacy of norgestomet ear implant for induction of estrus, restoration of ovarian cyclicity and fertility at induced and subsequent estrus during early (<90 days) and late (>120 days) postpartum periods. The suppressive effect of progestagen treatment on largest follicles was visible from day 3 onwards, which regressed @ 0.68±0.05 mm/d. Consequently, the largest follicle diameter was minimum on day 9 of treatment. After eCG injection (day 9), dominant follicle (DF) grew rapidly to attain significantly larger size than the second largest follicle when DF approached ovulation (day 12). The maximum size of ovulatory follicle was 12.05±0.36 mm. Overall, 14 of the 15 treated buffaloes ovulated and in 13 animals a corpus luteum was observed 10 days later. The conception rates at induced estrus and overall up to third insemination were 66.7% (10/15) and 86.7% (13/15), respectively. In 13 animals, which got pregnant, mean number of inseminations per conception were 1.31. Our results showed that Crestar + eCG was an effective therapy for inducing estrus in postpartum anestrus Murrah buffaloes.

Key words: Buffalo, Crestar, Estrus induction, Postpartum anestrus

Reproductive failures in buffaloes account for more than half of all losses resulting from various disorders including diseases. A long calving interval is mainly due to delay in resumption of ovarian cyclicity during early postpartum period, which could partly be attributed to seasonality which buffaloes do exhibit to a certain degree (Barile *et al.* 1997). Various hormonal regimes have been tried for induction of ovarian activity in postpartum anestrus buffaloes. Favourable effects of GnRH and its analogues in inducing postpartum estrus were reported in buffaloes (Singh *et al.* 2009). Other hormonal preparations including syncromate-B (Misra *et al.* 2003), crestar (Malik *et al.* 2010), Progestogen and PMSG alone or in combinations (Kumar *et al.* 2010), GnRH-agonist or hCG in combination were also used. However, there are wide variations in induction of estrus and subsequent conception rates. Hence, the present study was undertaken to evaluate the efficacy of norgestomet ear implant for the induction of ovulatory estrus, restoration of ovarian cyclicity and fertility at the induced and subsequent estrus in anestrus buffaloes during early (<90 days) and late (>120 days) postpartum period.

MATERIALS AND METHODS

The study was conducted on 15 normally calved, 2 to 8 months postpartum, lactating and suckled Murrah buffaloes. These animals were divided into group 1 (<90 days postpartum, n=8) and group 2 (>120 days postpartum, n=7). The average age of animals at the start of experiment in group 1 was 59.3±3.5 months (range 47 to 76 months) whereas in group 2 it was 60.0±1.8 months (range 55 to 71 months). All animals were of first parity, except 1 animal in group 1 which was of second parity, and in good body condition with body condition score of over 2.5 on a scale from 1 to 5. Weaning was not practiced but suckling was restricted to twice daily just before the dams were hand milked.

Housing, feeding and management of animals: Animals were housed in half-walled sheds, well protected from inclement weather, regularly allowed wallowing and given shower bath before morning and evening milking. Concentrate allowance was calculated as per milk yield of individual buffalo. Animals were allowed grazing for 2 to 4 h after morning milking. Estrus detection was done by parading a teaser bull daily in morning and evening.

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Ultrasound scanning of ovarian activity: Ovarian follicular changes in experimental animals were monitored with a real time B-mode ultrasound scanner equipped with a convex array multifrequency transducer using frequency of 7.0 MHz. During each examination, ovarian maps were drawn to record size and position of follicles and presence of CL to facilitate sequential evaluation of follicular turnover. The follicles were characterized as small (≤ 4mm diameter), medium (>4 to 8 mm diameter) or large (>8 mm diameter). In each animal, 2 ultrasound examinations were made at 10 days' interval to confirm the absence of cyclic structures. The animals were then subjected to daily or alternate day ultrasound examinations during treatment. Subsequently, examinations were made at weekly intervals till confirmation of pregnancy or reestablishment of cyclicity.

Treatment: All (n=15) true anestrus buffaloes were treated with norgestomet ear implant (containing 3 mg norgestomet) and intramuscular injection (containing 3 mg norgestomet and 5 mg estradiol valerate on day 0 (day of start of treatment), 500 IU eCG on day 9, withdrawal of ear implant on day 10 and 16 μg GnRH analogue (buserelin) on day 12, followed by insemination at estrus detected by a teaser bull and aided by ultrasonography.

Statistical analyses: Characteristics associated with ovarian follicular profiles were analyzed with General Linear Model Procedures of the statistical software package SPSS for windows, version 9.0.1. Where 2 variables were involved, Student's t-test was used to evaluate the differences between means, while Duncan's Multiple Range Test was used for comparing more than two variables.

RESULTS AND DISCUSSION

In norgestomet treated buffaloes the day 0 largest follicle (LF) regressed @ 0.68±0.05 mm/day and the total duration of regression was 8.40±0.43 days. The LF diameter fluctuated during the treatment period, being lowest on day 9 and attaining the maximum size on day 12 (Table 1). The differences between LF diameters on day 0 and day 9, day 0 and day 12 as well as day 9 and day 12 were significant (P<0.05) in both the groups. Around day 10 of the treatment, the ovulating follicle (OF) showed divergence and the mean ovulation day was 12.93±0.12 (Table 2). The rate of growth of OF after eCG treatment was significantly higher (P<0.01) than the rate of growth before eCG. The eCG along with norgestomet might be useful as eCG leads to induction of behavioural estrus signs (Singh *et al.* 2004). Incorporation of estradiol or eCG at the end of a progestogen synchrony treatment might result in earlier onset and tighter synchrony of estrus.

A better estrus response and conception in buffaloes was observed with higher doses (700–1000 IU) of eCG (Younis *et al.* 1996). In the present investigation, 500 IU eCG yielded encouraging results. The physiological mechanism underlying the effectiveness of progestogen treatment in inducing follicular growth and ovulation in anestrus animals is not fully known. The treatment of anestrus buffalo with Crestar might mimic the transient increase in circulating levels of progesterone which is observed prior to first overt estrus during resumption of ovarian cyclicity in postpartum period indicating that progesterone is involved in the re-establishment of postpartum ovarian cyclicity. If progesterone is an important negative feedback hormone in the buffalo, then removal of ear implant should result in higher concentrations of circulating gonadotrophins and in turn follicular development should be enhanced.

Table 1. Largest follicle profiles (mean±SE) during crestar treatment in postpartum (<90 and >120 days) anestrus buffaloes

Largest follicle profiles	< 90 days postpartum (n=8)	> 120 days postpartum (n=7)	All treated (n=15)
Diameter (mm) at			
Crestar (day 0)	8.93^{b}±0.88	10.41^{bc}±0.28	9.74±0.52
During crestar (day 2–9)	9.30^{bx*}±0.55	11.26^{aby}±0.37	10.13±0.41
eCG (day 9)	6.80^{cx}±0.36	8.24^{dy}±0.43	7.47±0.35
Crestar out (day 10)	8.50^{bc}±0.42	9.60^{c}±0.35	9.01±0.32
GnRH (day 12)	11.83^{a}±0.41	11.73^{a}±0.19	11.78±0.25
Regression of LF present at the start of treatment			
Day of start	3.13±0.54	3.29±0.44	3.20±0.37
Duration (days)	8.50±0.68	8.29±0.44	8.40±0.43
Rate (mm/day)	0.60±0.04	0.78±0.06	0.68±0.05

Values with different superscripts (a, b, c, d in a column and x, y in a row) differ significantly (P<0.05, *P<0.01) ; start of treatment (crestar in), day 0.

In the present investigation the day of implant insertion (day 0) had a mean dominant follicle (DF) diameter of 9.74±0.52 mm but none of the follicles ovulated and rather they regressed probably under the negative feedback of the progestogen treatment. Exogenous progesterone suppresses the DF in a dose dependent manner when given during the growing phase (Sanchez *et al.* 1995). The suppressive effect of norgestomet on large follicles was visible around day 3 and it regressed at the rate of 0.68±0.05 mm/d (Table 1). After eCG injection the growth of DF was very rapid and difference in size of DF and second largest follicle (SLF) became significantly wide as the DF approached ovulation (Table 2). In cattle also, OF grew more rapidly (P<0.05) but persisted for a shorter period as compared to most of the non-ovulating DFs. Lohan *et al*. (2004) also observed that OF developed rapidly and its size difference as compared to SLF, became much more near the ovulation. The maximum size of OF in the present study (12.05±0.36 mm) is in consonance with Taneja *et al.* (1996) that on the day of estrus, the OF was the LF on either ovary.

In the present study, majority of the animals (11/15) showed behavioural estrus within 48 to 72 h of implant removal. Soon after the removal of implant (day 10), there

Table 2. Ovulating follicle profiles in crestar treated postpartum (<90 and > 120 day) anestrus buffaloes

Largest follicle profiles	< 90 days postpartum (n=7♦)	> 120 days postpartum (n=7)	All treated (n=14)
Day of emergence	2.86±0.62	3.71±0.44	3.29±0.41
Day of divergence	10.0±0.18	10.57±0.34	10.29±0.22
Day of ovulation	13.0±0.20	12.86±0.13	12.93±0.12
Maximum diameter (mm)	12.37±0.65	11.73±0.19	12.05±0.36
Duration of growth (days)			
Before eCG	$6.14\pm0.62^{a*}$	$5.29\pm0.44^{a*}$	5.71±0.41
After eCG	3.14 ± 0.24^{b}	2.86 ± 0.13^{b}	3.00±0.15
Total	9.29±0.60	8.14±0.43	8.71±.40
Rate of growth (mm/day)			
Before eCG	$0.62\pm0.05^{a*x*}$	0.95 ± 0.08^{ay}	0.79±0.07
After eCG	1.81 ± 0.13^{bx}	1.34 ± 0.09^{by}	1.57±0.10
Overall	1.03±0.07	1.09±0.07	1.06±0.05
Rate of growth (mm/day)			
eCG to crestar out (day 9–10)	1.89±0.27	1.73±0.28	1.81±0.20
eCG to GnRH (day 9–12)	1.74 ± 0.13^{x}	1.29 ± 0.12^{y}	1.53±0.11
Crestar out to GnRH (day 10–12)	1.66±0.18	1.14±0.17	1.42±0.15
Difference in diameter from SLF (mm)			
At eCG (day 9)	$0.87\pm0.38^{a*}$	$1.74\pm0.50^{a*}$	1.31±0.33
At GnRH (day 12)	6.39 ± 0.79^{b}	$5.36^{b}\pm0.97^{b}$	5.87±0.64

Values for a parameter with different superscripts (x, y in a row and a, b in a column) differ significantly (P < 0.05, *P<0.01) ;♦1 animal did not ovulate and became cystic; SLF, second largest follicle.

Table 3. Fertility profiles (mean±SE) of crestar treated postpartum (< 90 and > 120 days) anestrus buffaloes

Parameters	<90 days postpartum	>120 days postpartum	All treated
Postpartum interval to start of treatment (days)	63.20 $\pm6.10^{x*}$ (n=8)	234.29 $\pm22.53^{y}$ (n=7)	142.93 ±24.65 (n=15)
Postpartum interval to end of treatment (days)	75.20 $\pm6.10^{x*}$ (n=8)	246.29 $\pm22.53^{y}$ (n=7)	154.93 ±24.65 (n=15)
Interval from crestar out to ovulation (days)	3.14 ±0.24 (n=7†)	2.86 ±0.13 (n=7)	3.00 ±0.14 (n=14)
CLA at day 10 post AI (mm^2)	215.13 ±24.18 (n=6♦)	226.19 ±13.17 (n=7)	221.07 ±13.85 (n=13)
Interval from treatment to conception (days)	36.14 ±21.01 (n=7)	6.0 ±4.56 (n=6)	22.23 ±12.24 (n=13)
Postpartum interval to conception (day)	114.14 $\pm26.42^{x*}$ (n=7)	238.00 $\pm23.64^{y}$ (n=6)	169.85 ±25.94 (n=13)

Values with different superscripts (x, y) in a row differ significantly (P<0.05, *P<0.01); [y]1 animal did not ovulate and became cystic;♦1 animal had short cycle.

was a spurt in the growth of DF and it might have been because of low concentration of progesterone soon after implant removal and associated with enhanced follicular growth (Malik *et al.* 2010). eCG supplementation (day 9) further stimulated follicular development and maturation as eCG is known to increase blood estrogen and in turn leads to induction of behavioural estrus signs (Singh *et al.* 2004). El-Fadaly *et al.* (1994) reported 84% estrus response in anestrus and subestrus postpartum buffaloes treated with norgestomet ear implants followed by 1000 IU eCG on the day of implant removal, in agreement with the present investigation. However, higher estrus response (100%) in Crestar treated adult buffaloes was reported by Lohan *et al.* (2001).

Overall, 93.3% (14/15) buffaloes treated in the present study, ovulated (Table 3) as confirmed by sudden disappearance of large DF and subsequent formation of CL at the same place observed by ultrasonography. Out of 14 animals that ovulated, corpus luteum (CL) was observed in 13 (93%) animals during ultrasound examination after 10 days, which means that in one animal, CL regressed prematurely and it had a short cycle. Similarly, Abdoon *et al.* (1994) and Yadav *et al.* (2001) reported 100% ovulation rate with norgestomet treatment of anestrus buffaloes, supplemented with eCG @ 1000 IU and 600 IU at implant removal, respectively. Comparatively low ovulation rates of 88% and 89% have also been reported by Singh *et al.* (2004) and Abeygunawardena *et al.* (1995) respectively, in norgestomet treated postpartum anestrus buffaloes.

The conception rates (CR) at induced estrus and overall up to third insemination in the present study were 66.7% (10/15) and 86.7% (13/15), respectively (Table 4). These findings are in close agreement with Yadav *et al.* (2001) who reported CR of 66.7% (4/6) and 83.3% (5/6) to first and third insemination, respectively. Similarly, a CR of 73% (32/44) within 90 days of treatment by Abeygunawardena *et al.* (1995) has also been reported.

In the present study, the mean number of inseminations per conception was 1.31 (Table 4). Very low CRs of 17.4% and 11.1% at norgestomet induced estrus have also been reported by Misra *et al.* (2003) and Pant *et al.* (2002), respectively. Higher ovulation and pregnancy rates observed in the present investigation could also be attributed to the supplementation of norgestomet +eCG treatment with GnRH. Administration of GnRH helps in the release of pituitary gonadotrophins which might account for higher ovulation and conception rates.

The present study showed that under the cumulative effect of Crestar withdrawal and supplementary injection of eCG, the LF diameter showed significant increase before ovulation. Supplementary eCG helped in divergence and accelerated

Table 4. Fertility parameters of crestar treated postpartum (<90 and >120 days) anestrus buffaloes

Characteristics	Crestar <90 days	Crestar* >120 days	All crestar treated
Animals treated	8	7	15
Parity	1st (n=7) 2nd (n=1)	1st (n=7)	1st (n=14) 2nd (n=1)
Age at start of treatment (months)	59.25±3.53	60.00±1.80	59.60±2.06
Days PP at start of treatment	63.2±6.1	234.3±22.5	–
Pregnant at induced estrus	5/8 (62.5%)	5/7 (71.4%)	10/15 (66.7%)
Cyclicity after treatment	3/3 (100%)	2/2 (100%)	5/5 (100%)
Pregnant to 2nd AI	1/3 (33%)	1/2 (50%)	2/5 (40%)
Total pregnant, up to 3rd AI	7/8 (87.5%)	6/7 (85.7%)	13/15 (86.7%)
Induced estrus to next estrus	104.33±8.06 (n=3)	84.00±37.48 (n=2)	96.20±16.37 (n=5)
Treatment to conception	36.14±21.01 (n=7)	06.00±4.56^{x} (n=6)	22.23±12.24 (n=13)
PP to conception	114.14±26.42 (n=7)	*238.00±23.64^{x} (n=6)	–
PP to end of treatment	75.0±5.97 (n=8)	246.29±22.53 (n=7)	–
Inseminations/ conception	1.43 (n=7)	1.17 (n=6)	1.31 (n=13)

Values with different superscripts (x, y) in a row differ significantly (P<0.05).

*In crestar > 120 days group, the postpartum interval to conception (238 days) is shorter than PP interval to end of treatment (246 days), as the one animal of this group which did not become pregnant had PP interval of 320 days at the start of treatment. So when it is excluded from the mean of PP interval to conception, the mean appears less.

growth of the DF, which emerged during early part of Crestar treatment. The results of present study indicated that norgestomet +eCG therapy was effective in inducing estrus in postpartum anestrus buffaloes and got satisfactory conception rates at induced estrus.

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