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Fertility response using three estrus synchronization regimens in lactating anestrous buffaloes

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ABSTRACT

The objective of present study was to elucidate the efficacy of 3 estrus synchronization regimens for optimizing fertility in lactating anestrous buffaloes. Regimen1 (9, GPG), buffaloes were administered (i/m) gonadotropin releasing hormone (GnRH, 0.02 mg) on day 0 and 9, and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 500µg cloprostenol sodium) on day 7; regimen 2 (17, GPG+CIDR), in addition to regimen 1, buffaloes were inserted per-vaginally a controlled internal drug release (CIDR, 1.38g progesterone) device for 7 days (day 0–7); regimen 3 (14, GP-hCG), in this regimen, the second-GnRH of the GPG regimen was replaced by human chorionic gonadotropin (hCG, 3000 IU, i/m). Artificial insemination was carried out on days 10 and 11 at 24 h interval. Buffaloes failing to conceive and returning to estrus were again inseminated at their spontaneous estrus. Subsequent to first-GnRH, with or without CIDR device, a good synchrony in the stage of estrous cycle on day 7 was evident as the diameter of largest follicles was not less than 10 mm and was similar between regimens. On day 10, the diameter of largest follicle was more in buffaloes synchronized with CIDR. Buffaloes administered a CIDR device exhibited better estrus behaviour at 48 (day 9) and 72 (day 10) h after CIDR withdrawal compared to controls (GPG). First service conception rate and total % of buffaloes conceived were higher in the buffaloes of GPG+CIDR regimen compared to other regimens. Treatment with CIDR withdrawal had no additional beneficial impact on plasma estradiol at induced-estrus. The body condition score, lactation status, thyroid hormone status and blood biochemical (glucose, total cholesterol, triglycerides, total proteins, creatinine and blood urea nitrogen) profiles were similar between buffaloes of various regimens. In brief, GPG+CIDR based synchronization regimen followed by AI at 72 h and 96 h after CIDR withdrawal is most suitable for optimizing the fertility status of lactating anestrous buffaloes.

Key words: Anestrus, Buffalo, CIDR, Fertility response, Ovsynch, Progesterone

The occurrence of anestrus (true anestrus and subestrus), and its alleviation remains an unresolved issue (Ghuman *et al*. 2008). Recently conducted transrectal sonographic studies revealed that anestrous buffaloes display follicular turnover in a typical wave like pattern with dominant follicles attaining 12.2±0.3 mm diameter (range: 10.1–15.7 mm, Ghuman *et al*. 2010c). The bovine dominant follicles of 10 mm diameter could be responsive to exogenous gonadotrophins as they acquire luteinizing hormone (LH) receptors (Wiltbank *et al.* 2002). Applying estrus synchronization regimens in anestrous buffaloes may provide a potential alternative for increasing their productive period (Ghuman *et al*. 2008). Estrus synchronization regimens used till date have achieved partial success with respect to conception rate in anestrous buffaloes (Baruselli *et al*. 2004, Ghuman *et al*. 2010a, 2010b, Singh *et al.* 2009a). This justifies the need to establish a hormone regimen that can consistently generate an acceptable conception rate in anestrous buffaloes irrespective of their ovarian status (true anestrus or subestrus). Thus, the objective of present study in lactating anestrous buffaloes was to evaluate specific ovarian, hormonal, and estrual events as well as subsequent conception rates associated with three different estrus synchronization regimens.

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MATERIALS AND METHODS

The study was conducted on 40 lactating anestrous buffaloes (age, 6.7±0.4 yr; body weight: 358.6±8.4 kg; body condition score (BCS), 3.01±0.1 (at 1–5 scale; Edmondson *et al.* 1986); parity 2.63±0.2, days from last calving, 283.5±21.9 days; milk yield, 5.14±0.4 l/day; suckled buffaloes, 47.5%) reared at private dairy farms. Buffaloes were kept in loose housing system and were fed chaffed green fodder, wheat straw, concentrates, mineral mixture and *ad lib.* drinking water. History of buffaloes has suggested that these animals have never exhibited estrus since the last calving.

Synchronization regimens: Buffaloes were randomly submitted to 3 estrus synchronization regimens. In regimen 1, buffaloes (control; 9) were subjected to ovsynch (GPG), which involved administration (i/m) of 20µg GnRH analogue (buserelin acetate) on day 0, followed by 500µg prostaglandin$F_{2\alpha}$ ($PGF_{2\alpha}$, cloprostenol sodium) on day 7, and a second-GnRH analogue treatment (20µg) on day 9. In regimen-2 (GPG+CIDR), 17 buffaloes were subjected to ovsynch plus controlled internal drug release (CIDR, 1.38g progesterone) regimen. In this regimen, CIDR was inserted per-vaginally for 7 days starting from day 0 till day 7 of GPG regimen. In regimen-3 (GP-hCG), 14 buffaloes were administered a modification of ovsynch in which the second dose of GnRH (on day 9) was substituted by a dose of human chorionic gonadotropin (hCG) (3000 IU, I/M).

Breeding: All the buffaloes were inseminated on day 10 and day 11 (at 9.00 am on each day, i.e. at 24 hrs interval). Buffaloes failing to conceive and returning to estrus were again inseminated at observed spontaneous estrus.

Ultrasonography: Transrectal ovarian ultrasonography was carried out on day 0 (day of first-GnRH), 7, 9 and 10. Ovarian sonography was done with a battery operated B-mode ultrasound scanner equipped with inbuilt interchangeable 5/7.5 MHz linear-array rectal transducer. The ovarian sketches were drawn and the size and relative location of largest follicle(s) and corpus luteum (CL) were recorded (Ghuman *et al.* 2010c). The ovulation was confirmed by disappearance of visible largest follicle (Ghuman *et al.* 2010c). Largest follicle growth rate (mm/day) was defined as the difference between the largest follicle diameters recorded on day 10 and on day 7, divided by three (Sà Filho *et al*. 2010).

Jugular vein blood sampling: Blood samples were collected on day 0, 7, 9 and 10 from the jugular vein in a heparinized vial. Plasma was separated immediately after blood collection and frozen at –20°C until analysis.

Estrus behaviour: Characteristics of induced-estrus were recorded between days 8 to 11. Buffaloes were observed for visual signs of estrus, twice per day for 30 min, by the same person. Scoring scale for observed symptoms of estrus and genital tract was followed (Singh *et al*. 2009b). Estrus score of each day of individual buffalo was summed up and mean estrus score was calculated for each day of a group.

Plasma analysis: Plasma progesterone was assayed with the solid-phase radioimmunoassay, using an indigenous progesterone polyclonal antibody (Ghuman *et al*. 2009). Sensitivity of the assay was 0.1 ng/ml; intra- assay and inter-assay variation coefficients were 6.5% and 9.2%, respectively. Plasma concentrations of 17β-estradiol (on day 9 and day 10) and thyroid hormones (total triiodothyronine-T3, total thyroxine-T4 and thyrotropin-TSH, on day 0 and day 10) were determined using commercially available ELISA kits. Plasma concentrations (on day 0 and day 10) of glucose, total cholesterol, triglycerides, total proteins, creatinine and blood urea nitrogen were determined colorimetrically with auto-analyzer using commercially available kits.

Conception rate: First and second service conception rates were noted by diagnosing pregnancy through rectal palpation on day 90 post-insemination.

Statistical analysis: Numerical data are represented as mean±S.E.M., and differences were considered significant at $P<0.05$. Chi-square ($\chi 2$) test (Dytham 1999) was employed for the number of buffaloes that responded to various treatments of different synchronization regimens and conceived subsequently. Two sample Student's t-test (Dytham 1999) was employed: (i) to compare plasma progesterone/ CL diameter of day 0 with day 7, and day 7 with day 9 and 10, and (ii) to assess differences in ovarian and endocrine parameters, estrus score and biochemical profile between buffaloes of various synchronization regimens. Statistical procedures were performed using MINITAB release 13.2 statistical software.

RESULTS AND DISCUSSION

Ovarian status at the start of synchronization regimens (day 0): The present study was carried out in a selected group of buffaloes despite showing, anestrous had largest follicle of ~10 mm diameter on day 0 (Table 1). In fact, previous observations have also suggested the development of dominant follicles in anestrous buffaloes (Ghuman *et al.* 2010c). Moreover, interpretation regarding luteal status (presence of CL and plasma progesterone >0.5 ng/ml) on day 0 (Table 1) revealed that at least 67.5% lactating buffaloes (n,27/40) were in subestrus and remaining were experiencing true anestrus.

Ovarian responses to first-GnRH (day 0) and CIDR placement (day 0–7): In response to first-GnRH, all the buffaloes ($P<0.05$) in different regimens indicated the appearance of a newly formed CL on day 7 (Table 1). However, it remained uncertain whether the largest follicle existing on day 0 had luteinized or ovulated in response to GnRH. The presence of higher plasma progesterone on day 7 in the CIDR-based regimen compared to remaining regimens ($P<0.05$, Table 1) could be due to supplementary contributions from the CIDR despite the release of progesterone from the CL (Table 1). In fact, progesterone-based intravaginal implants can maintain luteal phase plasma progesterone for a period of 15 days in true anestrous buffaloes (Singh *et al.* 2009a).

In an ovsynch regimen, the first-GnRH induces the release

Table 1. Ovarian response to different synchronization regimens in anestrous buffaloes

Parameters	Regimens	Day 0	Day 7	Day 9	Day 10
Largest Follicle diameter (mm)	1 (GPG, n=9)	10.5±0.7	10.7±1.1^{a}	13.7±0.8	14.6±0.8^{b,A}
	2 (GPG + CIDR, n=17)	9.4±0.6	12.8±1.5^{a}	15.6±0.8	17.7±0.7^{b,B}
	3 (GP-hCG, n=14)	11.0±1.0	12.9±0.7^{a}	15.3±0.7	17.7±0.9^{b,A}
Plasma Estradiol (Pg/ml)	1	-	-	415.8±207.6	390.8±174.9
	2	-	-	446.7±172.5	478.1±103.5
	3	-	-	456.9±130.3	640.5±129.0
Corpus Luteum diameter (mm)	1	10.1±1.3	9.4±2.3^{a}	7.2±1.7	3.7±1.7^{b}
	2	8.2±1.7	10.6±1.3^{a}	4.1±1.1^{b}	0.9±0.7^{b}
	3	7.4±1.4	9.8±0.9^{a}	2.5±0.9^{b}	0.0±0.0^{c}
Plasma Progesterone (ng/ml)	1	1.12±0.24	1.13±0.28^{a,A}	0.45±0.17	0.35±0.30^{b}
	2	0.73±0.18^{a}	2.21±0.32^{b,B}	0.15±0.04^{c}	0.11±0.04^{c}
	3	0.66±0.22	0.81±0.18^{a,A}	0.27±0.09^{b}	0.13±0.04^{b}
Estrous score	1	-	-	56.7±6.4^{b}	52.2±13.4^{b}
	2	-	-	92.3±14.0^{b}	88.2±15.1^{b}
	3	-	-	77.1±14.9^{b}	54.3±12.9^{b}

$^{a\ vs\ b,\ b\ vs\ c}$P<0.05, within row of a parameter;$^{A\ vs\ B}$P<0.05,within column of a parameter.

of LH from the pituitary, leading to ovulation and synchronous emergence of a follicular wave 1–2 days later (Ghuman *et al*. 2010b). In current study, subsequent to first-GnRH, with or without CIDR device, a good synchrony in the stage of estrous cycle was evident on day 7 in the buffaloes of various regimens. This was evident from the diameter of largest follicles (>10 mm) on day 7 following different regimens (P>0.05, Table 1). This observation is consistent with the previous reports in buffaloes (Neglia *et al*. 2003).

Ovarian responses to $PGF_{2\alpha}$ administration and CIDR withdrawal (day 7)

Luteal responses: In all the regimens, buffaloes responded (P<0.05) to a luteolytic dose of $PGF_{2\alpha}$ administered on day 7 as revealed by the decline in plasma progesterone and CL diameter during the subsequent period of 72 h (P<0.05, Table 1). Similarly, in previous studies, plasma progesterone had declined to basal concentrations within 2–3 days after $PGF_{2\alpha}$ treatment of mature CL (Dadarwal *et al*. 2009) or after CIDR withdrawal (Ghuman *et al.* 2010a).

Largest follicle development: Concomitant with the decline in plasma progesterone, the diameter of largest/dominant follicles observed in different regimens on day 7 continuously increased during the subsequent 72 h (P<0.05, Table 1). During this period (day 7–10), the growth rate of largest follicle was numerically higher (P>0.05) in the buffaloes of GPG+CIDR (1.77±0.37 mm/d), and GP-hCG (1.50±0.24 mm/d) regimens in comparison to GPG (control, 1.27±0.28 mm/d) regimen. Moreover, the diameter of largest follicle on day 10 was larger in buffaloes synchronized with CIDR device in comparison to buffaloes without CIDR (P<0.05, Table 1). The positive effect of progesterone on the follicular growth rate and diameter of the dominant follicle has been reported in bovines (Sà Filho *et al*. 2010).

Another potential problem with the synchronization regimens is the decrease in circulating estradiol prior to AI due to premature ovulation of the dominant follicles (Mann and Lamming 2000). Thus, it was also hypothesized that priming of reproductive axis with adequate plasma progesterone may stimulate the granulosa cells of dominant follicle to increase plasma estradiol (Sà Filho *et al*. 2010). On contrary, plasma estradiol around the time of synchronized estrus in GPG+CIDR regimen was similar to other regimens (P>0.05, Table 1).

Estrus behaviour (day 8-11) vis-à-vis AI at induced-estrus

Anestrous buffaloes administered with a CIDR device exhibited better estrus behaviour at induced-estrus compared to remaining regimens (GPG and GP-hCG), as suggested by the numerically higher (P>0.05) estrus score in buffaloes of GPG+CIDR regimen on the day of second-GnRH (day 9) and 24 h later. Thus, based upon the estrus score, higher proportion of buffaloes in GPG+CIDR regimen required AI on the day of second-GnRH compared to buffaloes subjected to remaining regimens (P<0.05, Table 1). The observation of estrus score could suggest that CIDR-based regimen was better for anestrous buffaloes. The priming of hypothalamo-hypophyseal pituitary gonadal (HPG) axis with adequate amounts of progesterone is beneficial for the recovery of HPG axis function and, hence a better display of estrus behaviour by anestrous buffaloes at induced-estrus (De Rensis *et al*. 2005).

In previous studies, due to the unpredictability of the timing of ovulation, buffaloes that used progesterone device were inseminated 72 h and 96 h later to achieve 51% conception rate (Barile *et al*. 2001). In current study, although estrus score was high (P<0.05,) on the day of second-GnRH/hCG but only a single buffalo from each regimen ovulated within 24 h of second-GnRH, thus suggesting that buffaloes of all the three regimens may require AI only at 72 h and 96 h after CIDR withdrawal or at 24 h and 48 h after second-GnRH/hCG.

Impact of synchronization regimens on conception rate

It was suggested that anestrous buffaloes respond poorly to ovsynch protocol, thus, yielding poor conception rates, compared to cyclic buffaloes (De Rensis *et al*. 2005). Therefore, attempts were made to improve conception rates in anestrous buffaloes by the addition of an intravaginal progesterone device to ovsynch protocol (De Rensis *et al*. 2005). In the present study, the % of first service conception rate was 44.4, 70.6 and 50.0 in regimen 1, 2 and 3, respectively. The % of buffaloes conceived in the GPG+CIDR regimens were higher (P<0.05) compared to GPG and GP-hCG regimens. These results indicate that sufficient priming of hypothalamic system with progesterone during the pre-conception period may be necessary for the optimal growth of ovulatory follicle, subsequent CL as well as for the priming of endometrium to enhance the conception rate (Stevenson *et al*. 2006).

Another objective of the present study was to evaluate the impact of replacing second-GnRH of ovsynch protocol with hCG on the conception rate of anestrous buffaloes. This was based upon the hypothesis that hCG has long half life and its dissociation from the LH receptors is gradual, thus promoting ovulation as well as differentiation of granulosa cells to highly steroidogenic CL during post-AI luteal phase (Schmitt *et al*. 1996). Unexpectedly, no beneficial impact on % of buffaloes conceived in GP-hCG regimen was observed in comparison to GPG regimen (P>0.05). Similarly, in previous studies, conception rates were similar following replacement of GnRH with LH or hCG (Berber *et al*. 2002, Carvalho *et al*. 2007).

The buffaloes that failed to conceive at induced-estrus in different synchronization regimens exhibited spontaneous onset of estrus in the subsequent 16–35 days. However, a high proportion of buffaloes of CIDR-based regimen exhibited estrus after failure of conception at induced-estrus compared to other regimens (P<0.05).

Impact of BCS, lactation status, thyroid hormone status and blood biochemical profile on the success of synchronization regimens

Previous observations have recommended that anestrous buffaloes considered for estrus synchronization should have optimal health and nutritional status (Neglia *et al*. 2003). Therefore, to avoid the negative effect of body condition and lactation status, buffaloes selected for the current study had optimal body weight, BCS and lactation status that was similar between various regimens (P>0.05). Moreover, in the current study, plasma concentrations of thyroid hormones (T3, T4 and TSH) and various biochemicals (glucose, total cholesterol, triglycerides, total proteins, creatinine and blood urea nitrogen) were within the physiological range and were similar between groups at the start of synchronization regimens and at the time of AI (P>0.05). Thus, it can be inferred that fertility response observed in various regimens of current study were not influenced by their BCS, lactation status, thyroid hormone status and blood biochemical profile.

Progesterone-based hormone regimen (GPG+CIDR) followed by insemination at 72 (day 10) and 96 (day 11) h after CIDR withdrawal is most favourable for optimization of fertility status in the lactating anestrous buffaloes.

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