Comparison of ovsynch and progesterone-based protocol for induction of synchronized ovulation and conception rate in subestrous buffalo during low-breeding season

Ghuman, S.*; Honparkhe, M. and Singh, J.

Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141004, India

*Correspondence: *S. Ghuman, Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141004, India. E-mail: ghuman_s@yahoo.co.in*

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Summary

The objective of the present study was to compare the impact of ovsynch and progesterone-based ovulation synchronization protocol on ovarian response and conception in buffalo (n=19) exhibiting subestrus during low-breeding season (maximum ambient temperatures and relative humidity ranging from 36-45°C and 30-80%, respectively). Group I buffalo (n=10) were administered ovsynch protocol (d 0 and d 9, 20 µg Buserelin acetate; d 7, 500 µg Cloprostenol sodium; i.m.) followed by AI on days 9 and 10. During the same period, another group of buffalo (n=9) were administered intravaginal progesterone (1.38 g) for 10 days along with the administration (i.m.) of 500 µg Cloprostenol sodium on day 9 and 20 µg Buserelin acetate on day 11, followed by AI on days 12 and 13. With ovsynch, all the buffalo ovulated in response to 1st GnRH and had functional CL (plasma progesterone, 1.61±0.23 ng/ml; corpus luteum, CL, 11.36±0.67 mm) on day 7. Thereafter, subsequent to 2nd GnRH, five buffalo ovulated within 24 h and the remaining five between 24 to 48 h. In comparison, with progesterone-based protocol, a better synchronization of ovulation ($P<0.05$) was observed as seven buffalo ovulated between 24 and 48 h and the remaining two between 48 and 72 h following GnRH administration. Moreover, in comparison to ovsynch, conception rate was better with progesterone-based protocol (30 vs. 66.7%; $P<0.05$). In summary, progesterone-based protocol was superior to ovsynch for synchronization of ovulation and subsequent conception rate in buffalo exhibiting subestrus during the low breeding period.

Key words: Subestrus, Buffalo, CIDR, Conception rate, Ovsynch

Introduction

Buffalo is a polyestrous animal displaying tendency towards seasonality of reproductive activity. The breeding season of buffalo starts in rainy period and winter is the most favorable period, while summer appeared to be the most unfavorable period for buffalo reproduction (Sule *et al*., 2001). Moreover, during summer, the majority of non-pregnant buffalo (77%) in rural areas remain in anestrus (Singh *et al*., 1989). Various hormone protocols used in anestrous buffalo have achieved partial success with respect to synchronization of ovulation and first service conception rate (Ghuman *et al*., 2009b; Singh *et al*., 2009; Ghuman *et al*., 2012). The use of progesterone-release intravaginal device (PRID) in an ovsynch protocol was instrumental for the improvement of conception rate in non-cyclic buffalo, although no significant improvements were observed in cyclic buffalo (Presicce, 2007). Moreover, with this modification, the conception rate increased from 19 to 30%, but remained still low compared to breeding season (45%; De Rensis and Lopez-Gatius, 2007). The subestrous buffaloes, which are known to exhibit ovarian cyclicity without any external signs of estrus, usually have low plasma progesterone concentrations compared to buffalo exhibiting overt estrus (Mondal and Prakash, 2002). Therefore, the present study was planned to establish a hormone protocol for subestrous buffalo for achieving consistent synchronization of ovulation followed by an acceptable fertility, especially during low-breeding season.

Materials and Methods

Animals

The study was conducted on nineteen buffaloes (age: 3-6 years, BCS: 3-4) reared at private dairy farms, Ludhiana (latitude: 30°56' N, longitude: 75°52' E), India, during the hot-humid months from June to August (referred to as the 'summer season' with maximum ambient temperatures and relative humidity ranging from 36 to 45°C and 30 to 80%, respectively). Buffalo were kept in loose housing system and were fed chaffed green fodder, wheat straw, concentrates, mineral mixture and *ad libitum* drinking water. History of the animals suggested their failure to exhibit estrus during the three-month period before the start of study. For further confirmation, all the buffalo were subjected to visual estrous detection, twice daily, for a month before the application of hormonal protocols. Before the start of study, ultrasound scanner was used at two time points separated by 10 days, to establish the ovarian status of buffalo.

Protocols

In group I, ten subestrous buffalo were subjected to ovsynch protocol that involved the administration (i.m.) of 20 µg GnRH analogue (Buserelin Acetate, Receptal® VET, Intervet India Private Ltd., India) on day 0, followed by 500 µg Prostaglandin $F_{2\alpha}$ analogue ($PGF_{2\alpha}$, Cloprostenol sodium; Vetmate™, Vetcare, India) on day 7, and a 2nd GnRH (20 µg) on day 9. The buffalo were inseminated on days 9 and 10 (Fig. 1) (Ghuman *et al*., 2009b). In group II, subestrous buffalo (n=9) were inserted with controlled internal drug release (CIDR, 1.38 g progesterone) for 10 days along with administration of $PGF_{2\alpha}$ (500 µg) and GnRH (20 µg) on days 9 and 11, respectively. These animals were inseminated on day 12 and 13 (Fig. 1) (Ghuman *et al*., 2012). One person did all the artificial insemination (AI) after assessing the microscopic picture of semen, which was collected from one bull.

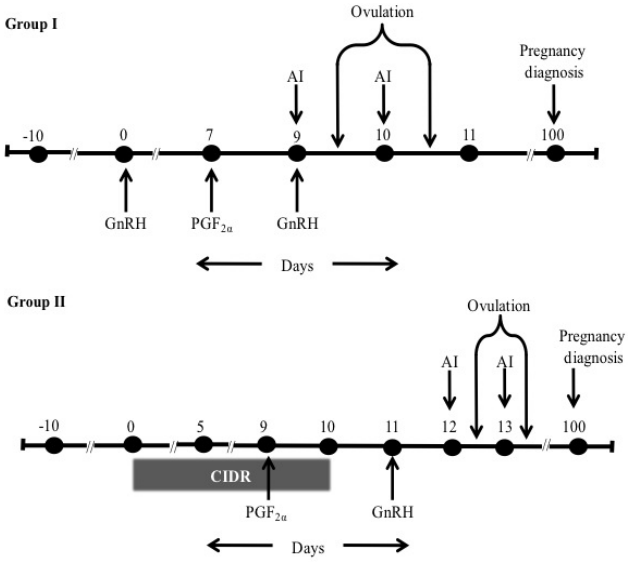

Fig. 1: Work plan of ovsynch (group I, n=10) and progesterone-based (group II, n=9) protocol in buffalo. AI: Artificial insemination, CIDR: Controlled internal drug release, GnRH: Gonadotropin releasing hormone, and $PGF_{2\alpha}$: Prostaglandin $F_{2\alpha}$

Ultrasonography

Transrectal ovarian ultrasonography was carried out on days 0, 7, 9, 10 and 11 in group I and on days 0, 10, 12 and 13 in group II (Fig. 1). Ovarian ultrasonography was carried out with a battery operated B-mode ultrasound scanner (Agroscan AL, ECM, Angouleme, France) equipped with inbuilt interchangeable 7.5 MHz linear-array rectal transducer (ALR 575 probe, ECM, Angouleme, France). Optimal scan images were frozen and the diameters of the largest follicles and corpus luteum (CL) were determined at their widest poles. All measurements were made using the built-in, on-screen calipers. The day when largest follicle disappeared was considered as the day of ovulation, that was further verified based upon the subsequent emergence of a CL on the site previously occupied by the disappeared largest follicle (Ghuman *et al*., 2010).

Jugular vein blood sampling and hormone analysis

Blood samples, in a heparinized vial, were collected on days 0, 7, 9 and 10 in group I and on days 0, 5, 9, 10 and 12 in group II (Fig. 1). Plasma was separated immediately and frozen at -20°C until the analysis of plasma progesterone with solid-phase radioimmunoassay, using a progesterone antibody raised in our laboratory (Ghuman *et al*., 2009a). Sensitivity of the assay was 0.1 ng/ml; intra-assay and inter-assay variation coefficients were 6.5% and 9.1%, respectively.

Fertility response

First service conception rate was recorded by diagnosing pregnancy through rectal palpation on day 100, subsequent to start of the synchronization protocols.

Statistical analysis

Numerical data are represented as mean ± SEM, and differences were considered to be significant at $P<0.05$. Fischer exact test of $\chi 2$ test was employed for the ovarian responses and subsequent conception of buffalo. Two sample Student's t-test compared the differences in plasma progesterone, and diameters of largest/ovulatory follicle and CL on different days of protocol. These statistical procedures were performed using MINITAB release 13.2 statistical software (Minitab Inc., State College, PA, USA) as per established procedures (Dytham, 1999).

Results

On the day of submission of subestrous buffalo to ovsynch (group I) or progesterone-based (group I) protocol, the mean diameter of largest follicles was 9.74 ± 1.09 mm and 11.78 ± 1.12 mm, respectively (Fig. 2). Moreover, these buffalo had active luteal profile at the start of hormone protocols (group I: CL, 7.20 ± 2.61 mm, progesterone, 1.10 ± 0.20 ng/ml; group II: CL, 7.47 ± 1.97 mm, progesterone, 0.90± 0.30 ng/ml; Fig. 2).

Ovulatory response

In ovsynch protocol (group I, Fig. 2), all the buffalo ovulated in response to first-GnRH as indicated by the appearance of functional CL on day 7 (CL, 11.36 ± 0.67 mm; progesterone, 1.61 ± 0.23 ng/ml). Moreover, the CL of all the buffalo responded to a luteolytic dose of $PGF_{2\alpha}$ on day 7 as indicated by the decrease in luteal activity on day 10 (CL, 7.16 ± 1.61 mm; progesterone, 0.38 ± 0.21 ng/ml; $P<0.05$). Subsequent to 2nd GnRH, all the buffalo ovulated either within 24 h (n=5/10) or between 24-48 h (n=5/10).

In progesterone-based protocol (group II, Fig. 2), there was a substantial increase in plasma progesterone on day 5 after CIDR insertion (0.94 ± 0.22 vs. 1.94 ± 0.26 ng/ml, $P<0.05$). About 48 h after CIDR removal, plasma progesterone reached basal concentrations (0.81 ± 0.12 vs. 0.14 ± 0.05 ng/ml; $P<0.05$). Following GnRH administration, all the buffalo ovulated either in the next

24 to 48 h (n=7/9) or between 48 to 72 h (n=2/9).

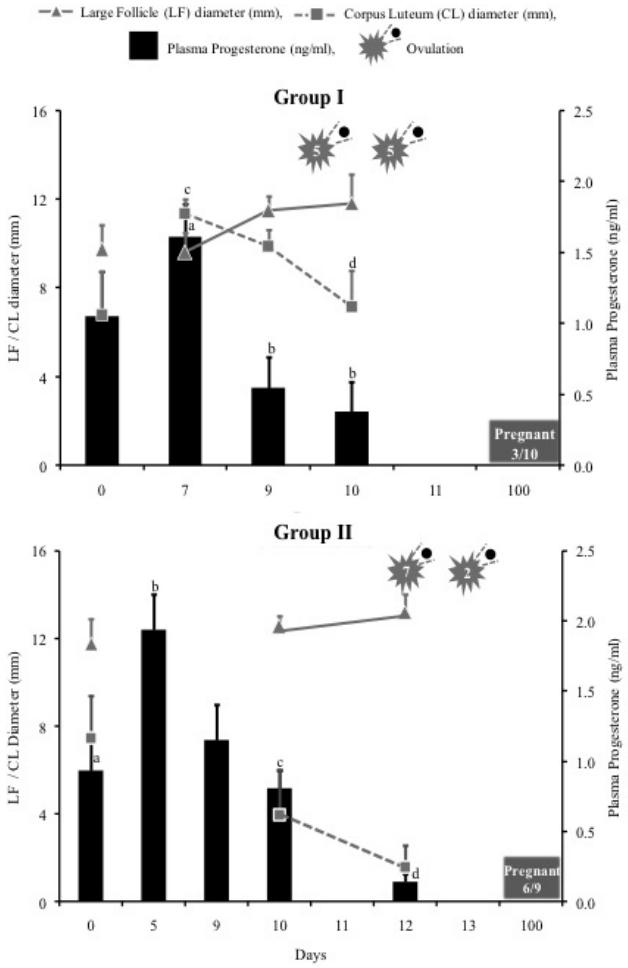

Fig. 2: Ovarian and fertility response in buffalo subjected to ovsynch (group I, n=10) or progesterone-based (group II, n=9) protocol. [a vs. b, c vs. d] $P<0.05$

Fertility response

First service conception rate of group I and II was recorded as 30% and 67%, respectively. Detailed retrospective analysis of data suggested that in group I, the buffalo that conceived subsequently had higher plasma progesterone at the start of protocol in comparison to their non-conceiving counterparts (2.23 ± 0.29 vs. 0.55 ± 0.24 ng/ml; $P<0.05$), but no difference ($P>0.05$) was observed in their largest follicle diameter either on the day of start of protocol or the ovulatory follicle diameter on the day of AI. Moreover, in group II, the ovulatory follicle diameter in buffalo destined to be non-pregnant or pregnant was similar ($P>0.05$).

Discussion

The history of buffaloes accompanied by their failure to exhibit estrus during three-month observation period and sonography at two time points separated by 10 day interval revealed the subestrous phase of buffaloes. In addition, the ovarian status viz, largest follicle or CL diameter as well as circulating plasma progesterone of buffalo at the start of hormone protocols confirmed that buffalo were in subestrous phase.

The ovulatory response of buffalo to 1st GnRH of ovsynch protocol was higher compared to previous studies (88%, Gumen *et al*., 2003). The decline in plasma progesterone to basal concentrations within 2-3 days after $PGF_{2\alpha}$ treatment during ovsynch protocol was also recorded previously in buffalo (Dadarwal *et al*., 2009). Following 2nd GnRH, the occurrence of ovulation in 50% buffalo within 24 h was lower compared to previous reports (78-90%; Baruselli, 2001; Paul and Prakash, 2005), where majority of the buffalo had ovulated around 23.3 ± 1.3 h (range: 20-32 h) after 2nd GnRH (Paul and Prakash, 2005). Nevertheless, subsequent to CIDR removal, ovulation of the largest follicle observed in all the buffalo was in accordance with previous studies in anovular cattle (Gumen and Wiltbank, 2005) and buffalo (Singh *et al*., 2009).

In progesterone-based protocol, the occurrence of better ovulation synchronization compared to ovsynch (78 vs. 50%) could be due to adequate progesterone priming of hypothalamic system, which is required for optimal growth of ovulatory follicle and synchronized LH surge (Rosenberg *et al*., 1991). In fact, the results of the present study with regard to maintenance of plasma progesterone around 1.0 ng/ml during the period of CIDR placement confirmed that placement of CIDR for 10 days was able to maintain plasma progesterone comparable to diestrus phase of cycling buffalo (Presicce *et al*., 2005; Dadarwal *et al*., 2009). In another study, progesterone-based intravaginal implant maintained luteal phase plasma progesterone for a period of 15 days in true anestrous buffalo (Singh *et al*., 2009). In buffalo receiving progesterone-based protocol, the presence of considerable plasma progesterone on the day of CIDR removal could be due to CIDR as the CL may not be functional on day 10 due to administration of $PGF_{2\alpha}$ to all the buffalo about 24 h before CIDR removal.

The conception rate observed in ovsynch-treated buffalo (30%) was marginally higher compared to previous studies in buffalo during low-breeding season (7-18%; Baruselli, 2001; Ghuman *et al*., 2009b). Nevertheless, compared to ovsynch-treated buffalo of the present study, the conception rate following progesterone-based protocol was higher and was comparable to a previous PRID-based estrus synchronization study in buffalo in which conception rate was 60% (Singh *et al*., 2009). This establishes the importance of progesterone priming during pre-conception period on subsequent conception rate (Stevenson *et al*., 2006). Moreover, ovsynch-subjected buffalo that conceived subsequently had higher plasma progesterone at the start of ovsynch in comparison to their non-conceiving counterparts.

Literature has suggested that a larger preovulatory follicle may generate a larger CL to secrete more progesterone and hereby have a positive effect on pregnancy rates (Binelli *et al*., 2009). In contrast, others reported absence of correlation (Colazo *et al*., 2009), negative correlation (Lynch *et al*., 2010) or positive

correlation (Pandey *et al*., 2011) between preovulatory follicle diameter and pregnancy outcome. In the present study, in buffalo that subsequently conceived or failed to conceive, there was no difference in the ovulatory follicle diameter on the day of AI, both in ovsynch and progesterone-based protocol. Nevertheless, our observations suggested that large follicles present during critical periods of ovsynch protocol were at similar stages of growth and were functional, thus ovulated (Kastelic *et al*., 1990).

In brief, although both ovsynch and progesterone-based protocol were successful for inducing ovulation, the timing of ovulation was better synchronized following progesterone administration. Furthermore, the importance of plasma progesterone during pre-conception period on subsequent fertility was shown in subestrous buffalo during low-breeding season.

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