INDUCTION OF OVULATORY ESTRUS USING OVSYNCH PROTOCOL AND SUBSEQUENT FERTILITY IN TRUE ANESTRUS BUFFALO HEIFERS

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ABSTRACT

The aim of using Ovsynch protocol in true anestrus buffalo heifers (n=11) was to induce synchronized ovulatory estrus and thereafter, to assess the first service conception rate. 'Ovsynch' involved administration of a GnRH-analogue at days 0 and 9, and a $PGF_{2\alpha}$ analogue on day 7. Transrectal ovarian ultrasonography and jugular vein blood sampling was done daily starting from first-GnRH till ovulation after second-GnRH. Thereafter, these procedures were performed on days 5, 10, 15, 21 and 42 post-ovulation. Heifers were inseminated on day 10 and if required on day 11. In response to first-GnRH, all the heifers ovulated ($P<0.05$) within 4 days (1.82±0.44 days) with subsequent development of corpus luteum (CL). All the heifers responded ($P<0.05$) to $PGF_{2\alpha}$ on day 7. Subsequent to second-GnRH (day 9), all the heifers exhibited estrus symptoms ($P<0.05$) which disappeared in the event of ovulation ($P<0.05$) between days 10 and 11 (n=9) or between days 11 and 12 (n=2). First service conception rate after Ovsynch protocol was poor (18%, $P<0.05$). Post-Ovsynch luteal profile showed comparable CL growth in all the heifers, except on day 21 and 42 ($P<0.05$-0.10) when CL was small in non-pregnant heifers. Plasma progesterone was sub-optimal on day 5 ($P<0.05$) in heifers that failed to conceive. In conclusion, Ovsynch protocol was 100% successful for inducing ovulatory estrus which was synchronized in 82% anestrus buffalo heifers, thus requiring insemination only at 24 h subsequent to second-GnRH. More studies should be planned to suggest strategies for improving the first service conception rate in anestrus buffalo heifers subjected to Ovsynch protocol during summer season.

Key words: Anestrus, Buffalo, Fertility, Ovsynch, Ovulation

INTRODUCTION

Reproductive efficiency of buffaloes is limited by the occurrence of true anestrus (Ghuman *et al.*, 2008a). Using transrectal ultrasonography, it was recently revealed that during true anestrus, all the buffaloes display variable degree of ovarian activity. This was characterized by follicular turnover in a typical wave like pattern with follicles >9 mm found in one or both ovaries. However, the dominant follicles regressed without ovulation even after attaining ovulatory size (Ghuman *et al.*, 2008b). Such true anestrus buffaloes with large follicles may respond to the use of exogenous gonadotropins (Ghuman *et al.*, 2008a). These observations advocated the use of Ovsynch protocol in anestrus buffaloes to induce synchronized ovulation of large follicles. Ovsynch has already found application with encouraging results in cyclic buffaloes (Paul and Prakash, 2005). Thus, the aim of the present study in anestrus buffalo heifers was: a) to monitor dominant follicle and corpus luteum (CL) development during Ovsynch program, and b) to observe post-Ovsynch estrus response, ovulation time, luteal profile and fertility.

MATERIALS AND METHODS

Present experiment was conducted on buffalo heifers (Age: 3-4 years, Body weight: 402.5±15.0 kg, BCS: 3-4) reared at the dairy farm of Guru Angad Dev Veterinary and Animal Sciences University. Heifers were kept in loose housing system and were fed chaffed green fodder, wheat straw, concentrates (maize or wheat 60%, groundnut cake 25%, wheat bran 10%, rice bran 5% and common salt 1%), mineral mixture and *ad libitum* drinking water. Heifers were allowed to wallow in a pond of water twice in a day for 30-60 min. The period of experiment was summer (December-August) when ambient temperature and relative humidity were ranging between 36-45°C and 30-90%, respectively.

Treatment : Eleven buffalo heifers selected for study had the history of anestrus as suggested by bull parading during 12 month period before the start of study. For further confirmation of true anestrus, ultrasonography of reproductive tract and plasma progesterone analysis was carried out at two time points, separated by 7 days, before the start of study. Thereafter, all the buffalo heifers were subjected to Ovsynch program. In brief, Ovsynch involved administration of 20µg of a GnRH analogue (Buserelin Acetate, Receptal® VET, Intervet India Private Ltd., Pune, Maharashtra, India) on day 0, followed by 500µg of Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, Cloprostenol sodium; Vetmate™, Vetcare, Bangalore, Karnataka, India) on day 7, and a second-GnRH treatment of same analogue (20 µg) on day 9. All treatments were administered by intramuscular route in the neck. All the heifers were inseminated on day 10 and on day 11, if required (based upon presence of ovulatory follicle). At the same time estrus response was noted based upon the vasectomised (teaser) bull parading, cervico-vaginal discharge and uterine tone. Inseminations were done by one person after properly checking microscopic quality of semen.

Ultrasonography and blood sampling : Transrectal ovarian ultrasonography and jugular vein blood sampling was carried out daily starting from the day of first-GnRH till the day of ovulation after second-GnRH. Subsequently, these procedures were performed on days 5, 10, 15, 21 and 42 post-ovulation.

Ovarian ultrasonography was carried out with a battery operated B-mode ultrasound scanner (Agroscan AL, ECM, Angouleme, France) equipped with inbuilt interchangeable 5/7.5 MHz linear-array rectal transducer (ALR 575 probe, ECM, Angouleme, France). Ovaries were systematically examined and images were recorded on a diagram of the ovary by carefully sketching the size and relative location of all follicles of >4 mm diameter and size and relative location of visible CL (Ghuman *et al.,* 2008b). Optimal scan images were frozen and the size of the follicles / CL was determined by measurement of the diameter of the follicles / CL at their widest poles. All measurements were made using the built-in, on-screen calipers. Blood was sampled from the jugular vein in a heparinized vial after each ultrasonography. Plasma was separated immediately after blood collection and frozen at -20°C until analysis.

The emergence of dominant follicle was the day when the dominant follicle was, retrospectively, identified at a diameter of 4-5 mm. The dominant follicle was the largest follicle in a follicular wave that was 2 mm greater than the second-largest follicle (Ghuman *et al.,* 2008b). The dominant follicle was considered ovulatory when the dominant follicle ovulated and was considered nonovulatory when regressed. The regression of dominant follicle was accepted when its maximal diameter started to decrease and was no longer detectable. The life span of ovulatory dominant follicle was from the day of emergence to the day of ovulation (Ghuman *et al.,* 2008b). The day when dominant ovulatory follicle disappeared was the day of ovulation. Ovulation was verified based upon the subsequent emergence of a CL on a site previously occupied by the disappeared dominant follicle (Ghuman *et al.,* 2008b).

Fertility response : First service conception rate was noted by diagnosing pregnancy on day 42 subsequent to second-GnRH induced ovulation on non-return basis.

Hormone analysis: Plasma progesterone was assayed with the solid-phase radioimmunoassay (Kamboj and Prakash, 1993), using a progesterone antibody raised in our laboratory (Ghuman *et al.*, 2009). Sensitivity of the assay was 0.1 ng/ml; intra- assay and inter-assay variation coefficients were 6.7% and 8.9%, respectively.

Statistical analysis: Chi-square (+2) test (Dytham, 1999) was employed for the number of heifers that responded to various treatments of Ovsynch and conceived subsequently. Two sample student's t-test (Dytham, 1999) was employed to compare plasma progesterone, CL / ovulatory diameter on different days during Ovsynch and post-Ovsynch period between heifers that conceived or failed to conceive. Statistical procedures were performed using MINITAB release 13.2 statistical software.

RESULTS AND DISCUSSION

Confirmation of true anestrus : Prior to application of Ovsynch in anestrus buffalo heifers, the mean diameter of largest follicles was 11.50±0.25 mm. This was in accordance with our previous observations in anestrus buffalo heifers (Ghuman *et al.*, 2008b). These large follicles fails to ovulate and regress (Ghuman *et al.*, 2008b), as suggested by the absence of CL and presence of meager plasma progesterone (0.15±0.01 ng/ml) as observed at weekly interval before the start of study. This confirmed that buffalo heifers were in true anestrus.

Display of estrus during post-Ovsynch period: All the anestrus buffaloes responded (P<0.05) to Ovsynch program by displaying estrus symptoms viz. response to teaser bull, and presence of cervico-vaginal discharge and uterine tone. Subsequent to administration of second-GnRH, these estrus symptoms persisted for 24 h (n=9 heifers) or 48 h (n=2 heifers). Thus, Ovsynch program successfully induced synchronized estrus response in 82% heifers (P<0.05). At synchronized estrus, all the heifers were inseminated, but those which failed to conceive (n=9) exhibited spontaneous estrus at 20-24 days interval. This suggested that regular estrus cyclicity was initiated in true anestrus heifers subsequent to administration of Ovsynch. Underlying mechanism for the induction of estrus could be priming of hypothalamus with progesterone and estrogen. During Ovsynch, the former is secreted from the CL which appears subsequent to first-GnRH induced ovulation, whereas, the latter is released from the mature dominant follicles present at the time of second-GnRH.

Response to first-GnRH : In response to first-GnRH (day 0), all the heifers ovulated (P<0.05) within 4 days (Table 1). This response was higher compared to previous studies in anovulatory cattle (88%, Ghuman *et al.*, 2003). Mean diameter of ovulatory follicles on day of ovulation was larger (P<0.10) than their diameter on day 0 (Table 1). This is due to the fact that on day 0, all the heifers exhibited follicles >9 mm which ovulated within 2 days, except in two heifers. In these heifers, the large follicles regressed and small follicles (~4.5mm) started to grow and ovulated. This can be explained based upon the findings that on the day of first-GnRH, large follicles present in their growing phase were functional and ovulated, but such ability might have been lost during the late plateau or regression phase of development of follicles (Silcox *et al.*, 1993) in some heifers. On day 7, all the heifers had CL as well as higher (P<0.05) plasma progesterone compared to day 0 (Table 1). This confirmed the response to first-GnRH (Ghuman *et al.*, 2003). However, mean age of CL on day 7 was variable (Table 1) due to variation in day of ovulation in response to first-GnRH.

Response to $PGF_{2\alpha}$: All heifers responded (p<0.05) to $PGF_{2\alpha}$ as determined by decrease in CL diameter and plasma progesterone within 48 h (P<0.05, Table 1). This confirmed luteolysis at the time of second-GnRH. Similarly, in previous studies, plasma progesterone declined to basal concentrations within 2 days after $PGF_{2\alpha}$ treatment (Dadarwal *et al.*, 2009).

Response to second-GnRH : All the heifers ovulated (P<0.05) following second-GnRH on day 9 (Table 1). Moreover, in contrast to observations at the time of first-GnRH, no difference was observed between

the diameter of ovulatory follicle on the day of second-GnRH and on the day of ovulation (Table 1). These observations suggested that dominant follicles present at the time of second-GnRH had attained ovulatory size and were functional, thus ovulated (Kastelic *et al.*, 1990). Furthermore, ovulation was synchronized in 82% heifers (n=9/11), that was similar to previous reports in buffaloes (Paul and Prakash, 2005). In this study, ovulation time was between days 9 and 10 (n=9) or between days 10 and 11 (n=2). This was in accordance with previous studies in which majority of the buffaloes ovulated around 23.3±1.3 h (range: 20-32 h) after second-GnRH (Paul and Prakash, 2005). Moreover, ovulation rate in response to either first- or second-GnRH was 100% (Table 1). In previous studies, there was greater response to second-GnRH than first-GnRH (Gu¨men *et al.*, 2003).

Post-Ovsynch luteal profile and fertility : In heifers destined to be non-pregnant (n=2) or pregnant (n=9), the recording of similar diameter of ovulatory follicle (13.93±0.68 and 14.75±1.25 mm, respectively) was in contrast to previous studies (Ghuman *et al.*, 2003). Moreover, the development of CL till day 15 post-ovulation was similar in non-pregnant and pregnant heifers, whereas on days 21 and 42 post-ovulation, the CL was better developed in pregnant ($p<0.05$-0.10). It is well known that secretion of progesterone by the CL is essential for successful gestation (Shalam-Albalancy *et al.*, 1997). In fact, out of 9 non-pregnant heifers, 3 were in estrus by day 21 post-ovulation which might have suffered from failure of fertilization or early embryonic mortality, whereas remaining 6 were in estrus by day 42 post-ovulation suggesting late embryonic mortality. Suboptimal progesterone support during luteal phase causes insufficient priming of endometrium which may lead to early or late embryonic mortality (McNeill *et al.*, 2006).

Nevertheless, the first service conception rate (18%, $P<0.05$) in Ovsynch-treated acyclic buffalo heifers was comparable to that reported in previous studies

Table 1: Details of various variables related to induction of synchronized ovulation in anestrus buffalo heifers using Ovsynch protocol. Figures in parenthesis [..] indicate range. CL: corpus luteum, n: number, OF: ovulatory follicle, P_4: progesterone

Ovsynch protocol	Heifers ovulated (n)	Day of ovulation	OF diameter (mm)	Life span of OF (days)	Heifers with CL (n)	Age of CL (days)	CL diameter (mm)	Plasma P_4 (ng/ml)
Day 0: first-GnRH	-	-	10.75 ± 1.21^{c} [4.5-17.6]	-	-	-	-	0.16 ± 0.03^{a} [0.0-0.35]
1st-GnRH induced ovulation	11	1.82 ± 0.44 [0.0-4.0]	13.33 ± 0.80^{d} [9.8-17.6]	-	-	-	-	-
Day 7: $PGF_{2\alpha}$	-	-	-	-	11	4.91 ± 0.44 [3.0-7.0]	16.32 ± 0.50^{a} [12.9-18.4]	0.43 ± 0.10^{b} [0.0-1.0]
Day 9: 2nd-GnRH	-	-	13.32 ± 0.58 [10.8-16.6]	6.82 ± 0.52 [5.0-10.0]	-	-	10.01 ± 0.54^{b} [8.3-12.9]	0.03 ± 0.01^{a} [0.0-0.15]
Second-GnRH induced ovulation	11	1.18 ± 0.12 [1.0-2.0]	14.08 ± 0.59 [9.6-16.7]	-	-	-	-	-

$^{a\ vs\ b}p<0.05$, $^{c\ vs\ d}p<0.10$: within column

(7%) which were conducted in buffaloes during summer months (Baruselli, 2001). In addition, following Ovsynch, low conception rate reported in acyclic buffaloes can be increased (from 5 to 30%) if progesterone is added to the Ovsynch protocol between the first-GnRH and $PGF_{2\alpha}$ (De Rensis *et al.*, 2005).

In conclusion, this study explored the fact that Ovsynch protocol can be used to induce synchronised ovulatory estrus in anestrus buffalo heifers (100%). An insemination is required only at 24 h after second-GnRH in majority (82%) of the heifers. Investigations are required to have better first service conception rate after Ovsynch program in acyclic buffalo heifers during summer season.

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