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Fertility response following ablation-induced follicular wave emergence and ovulation induction in anestrous buffaloes

MRIGANK HONPARKHE[1]✉, VINOD KUMAR GANDOTRA[1], PARKASH SINGH BRAR[1], ASLOOB AHMED MALIK[2], DINESH DADARWAL[1] and JASWANT SINGH[3]

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141 004 India

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ABSTRACT

The fertility response following induction of follicular wave emergence by follicle ablation combined with progesterone based ovulation synchronization in postpartum anestrous buffaloes was assessed. Postpartum anestrous buffaloes (n=24) with absence of overt estrus signs categorized as treatment group (Group 1; n=15) and control group (Group 2; n=9). Group 1 was subjected to transvaginal ultrasound-guided follicle ablation on a random day of the estrous cycle (Day=0) and insertion of CIDR. Seven days later, CIDR was removed and prostaglandin $F_{2\alpha}$ analogue was given intramuscularly. GnRH analogue was injected on day 9.5 and fixed-time AI was done 12 h and 24 h later. Ultrasonography was performed to follow ovarian structures on different occasions and on day 38 post-ovulation for detecting pregnancy. Group 2 did not receive any treatment however, estrus detection in this group was done daily by bull parading and visual monitoring followed by AI if found in standing estrus. In treatment group, the follicular wave emergence occurred by 24 h following ablation in 60% buffaloes and in 40% remaining animals by 48 h. The estrus exhibition was 100 % in group 1 versus 22.2 % in group 2. Mean interval from CIDR removal to ovulation was 81.2±3.08 h and from wave emergence to ovulation was 9.92±0.18 days. The first service pregnancy rate in treatment group was higher than control (66.67% vs 22.2%). The induction of follicular wave emergence through ultrasound guided follicle ablation and progesterone based ovulation synchronization can be used to improve pregnancy rates in anestrous buffaloes.

Keywords: Anestrous, Buffalo, Follicle ablation, Ovulation synchronization, Progesterone device

The reproductive efficiency of buffaloes is mainly hampered by poor estrus expression i.e. silent estrus, seasonal (mainly nutrition) influences, prolonged post-partum ovarian quiescence/anestrus (Singh *et al.* 2000, Ghuman *et al.* 2010, Das and Khan 2010) and repeat breeding syndrome (Singh *et al.* 2009a). Therefore, optimal reproductive management of buffaloes require implementation of estrus and ovulation synchronization protocols. Conventionally, these protocols include use of prostaglandin, GnRH and progesterone, either alone or in combination and yield varying degree of fertility in normal and subfertile buffaloes (De Rensis and Lopez-Gatius 2007, Frares *et al*. 2013, Kalwar *et al.* 2015). Synchronizing the follicular wave emergence using follicular ablation, GnRH or estradiol administration in cattle (Mapletoft *et al*. 2003) and buffalo (Honparkhe *et al*. 2014, Bhat *et al*. 2015) provides a predictable ovulation timing and thus allows fixed-time artificial insemination without a need to detect estrus. Previous studies have used progesterone (CIDR, PRID, Crestar and progesterone injections) alone or in combination with GnRH to improve ovulation timings and fertility in anestrous and subestrous buffaloes (Singh *et al*. 2009b, Ghuman *et al*. 2012, Ghuman *et al*. 2015). However, the efficacy of follicular ablation along with progesterone device followed by GnRH to induce ovulation has not been assessed so far in anestrous buffaloes. Alike cattle, ablating antral follicles > 5 mm buffaloes at random stages of estrous cycle induce new follicular wave emergence within 1–2 days after ablation (Honparkhe *et al*. 2014). The present field trial was aimed to evaluate fertility response following ablation-induced follicular wave emergence at random stage of estrous cycle in CIDR-treated anestrous buffaloes compared to those inseminated at detected estrus. We hypothesized that combining follicular ablation with intravaginal progesterone device followed by GnRH administration will synchronize ovulation and improve pregnancy rates after fixed-time AI in treated buffaloes as compared to untreated ones.

Present address: [1]Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab. [2]Sher-e-Kashmir University of Agricultural Sciences and Technology, Kashmir, Jammu and Kashmir. [3]Western College of Veterinary Medicine University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5B4. ✉Corresponding author email: mhonparkhe @gmail.com

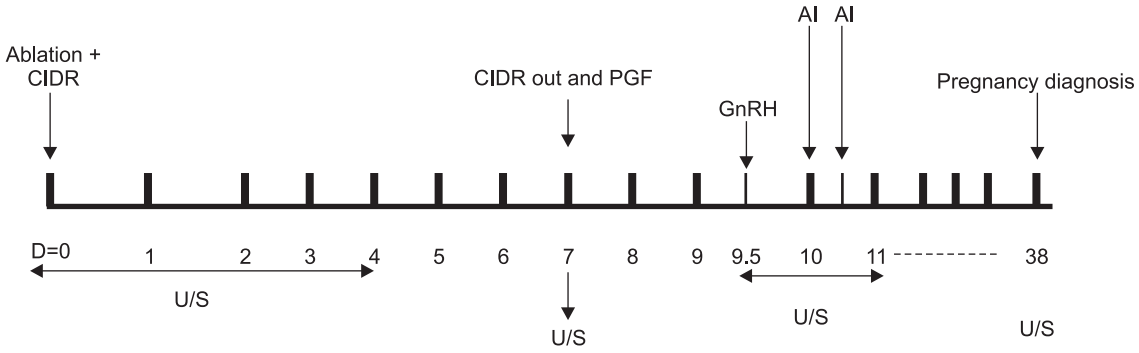

Fig. 1. Time line for the protocol used for the subfertile group buffaloes. D=0 is random day of estrous cycle when follicle ablation was carried out; CIDR, intravaginal progesterone releasing device; GnRH, gonadotropin releasing hormone analogue; AI, Artificial insemination; PGF, prostaglandin $F_{2\alpha}$ analogue; U/S, ultrasonographic examination of ovarian structures.

MATERIALS AND METHODS

The study was conducted on Murrah breed of buffaloes maintained at a dairy farm having herd size of 200 (during the period from October to December). Buffaloes (24) with the history of non-exhibition of estrus signs were selected for the study. These buffaloes were 90–180 days post-partum, lactating, weighed between 400–450 kg, having 2nd to 3rd parity and were reared in a loose housing system. The estrus detection method was bull parading combined with visual observation twice (morning and evening) at 12 h apart. These buffaloes were allocated to 2 groups, viz. treatment group (group 1; n=15) and untreated control group (group 2; n=9) and. Rectal palpation and transrectal ultrasonography (B-mode scanner Aloka SSD 500, equipped with 7.5 MHz linear-array transducer) were carried out in all the buffaloes 4–5 days prior to start of experiment to evaluate the ovarian and uterine status of the animals. All animals were non-pregnant as well as free from ovarian and uterine abnormalities. On the day of first observation, out of 24 buffaloes, corpus luteum (CL) was detected in 14 buffaloes (range: 7.0 to 13.2 mm) whereas 10 buffaloes had an ovarian follicle larger than 6 mm in diameter (range: 6.8 to11.3 mm).

In group 1 buffaloes, on a random stage of the estrus cycle, all follicles <5 mm on the ovaries were ablated by transvaginal ultrasound-guided follicle ablation procedure using Aloka SSD 500 and 5.0 MHz sector array end-fire transducer equipped with needle-guide (Bergfelt *et al*. 1994). On the day of ablation (Day 0), progesterone releasing intravaginal device (CIDR, 1.9 g, Pfizer Canada Inc., Saint-Laurent, Québec, Canada) was placed in the vagina (Fig. 1). Seven days later (Day 7), prostaglandin $F_{2\alpha}$ analogue (500 µg cloprostenol sodium, Vetmate, Vetcare India) was given intramuscularly and CIDR were removed. On day 9.5, GnRH analogue (20 µg buserelin acetate; Receptal, Intervet India) was given intramuscularly. All buffaloes were artificially inseminated with frozen semen from a bull with known fertility at 12 h and 24 h after the GnRH injection. All animals were subjected to transrectal ultrasonography from Day 0 to Day 4 (to confirm the day of wave emergence and development of a dominant follicle), then on Day 7 (i.e. day of CIDR removal), Day 10 (i.e. prior to GnRH Inj), Day 11 (to confirm ovulation), Day 16 (i.e. 5 days post-ovulation for the presence of CL) and 38 days post-ovulation for pregnancy status (Fig. 1). During each examination, ovarian sketches were drawn to record the location of all visible follicles and CL, and maximum diameters of ovarian structures were measured. The day of follicular wave emergence was determined retrospectively from ovarian drawings and defined as the day on which the dominant follicle was first identified at 4–5 mm in diameter. The day of ovulation was determined ultrasonically by the disappearance of a large follicle.

Group 2 buffaloes were observed daily for estrus signs (manual by an observer as well as bull parading) during period of the experiment and were subjected to AI if detected in standing estrus at 12 and 24 h after the start of estrus signs. The pregnancy status was detected in these buffaloes on Day 40 post-breeding through transrectal ultrasonography.

*Statistical analysis:*Numerical data are represented as mean±SEM. The diameter and growth rates for dominant versus subordinate follicles and diameter of CL in pregnant versus non-pregnant buffaloes of the subfertile group were analyzed by t-test. The diameter of dominant and subordinate follicles and the number of follicles in different size categories following wave emergence to Day 3 of wave were analyzed by repeated measures analysis within Proc mixed using compound symmetry (covariance structure) in SAS (Statistical Analysis Software) system. Pregnancy rates of the two groups are compared by chi-square test. A P-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The characteristics of ablation-induced wave are shown in Table 1 and Fig. 2. Majority of buffaloes (9/15; 60%) had wave emergence at 24 h after ablation whereas, remaining 40% (6/15) buffaloes had wave emergence at 48 h post-ablation. In 12 buffaloes, the largest subordinate

Table 1. Characteristics of ablation-induced follicular wave from wave emergence to ovulation and CL formation in the subfertile group of water buffaloes

Parameter	Mean (±SEM)	Range
Maximum diameter of dominant ovulatory follicle (mm; n=15)	11.3±0.52	8.5–14
Interval from CIDR removal to estrus exhibition (hours; n=15)	60.0±3.3	36–72
Interval from estrus to ovulation (hours; n=13)	48.0±3.8	24–72
Interval from CIDR removal to ovulation (hours; n=13)	81.2±3.08	72–96
Interval from wave emergence to ovulation (days; n=13)	9.92±0.18	9–11
Size of CL on day 5 post-ovulation (mm; n=13)	13.5±0.75	11–18.2

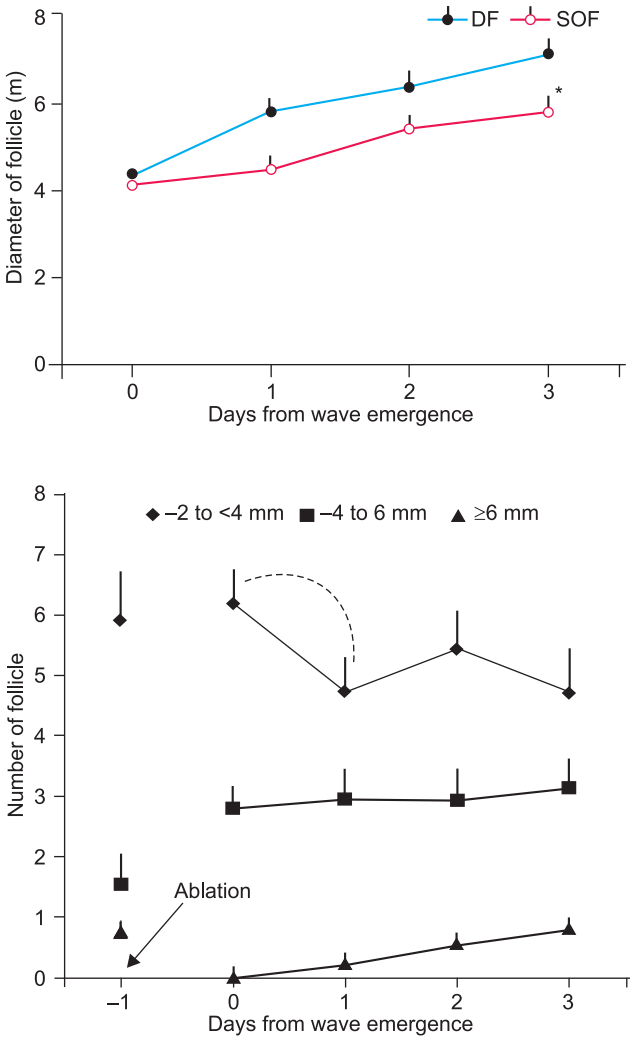

Fig. 2. Mean (±SEM) diameter of dominant (DF) and subordinate (SOF) follicle (Upper panel; n=15) and mean (±SEM) number of different sized follicle (lower panel) from day of wave emergence till Day 3 of cycle in group 1. *, P<0.05.

follicle was identified at 3–4.5 mm size on Day 0, while in others it was detectable on Day 1. The diameter of dominant follicle remained larger (P<0.05) than that of subordinate follicle from Day 1 to 3 of examination (Fig. 2; A and B, upper panel). The mean (±SEM) growth rate of dominant follicle was greater (P<0.05) than that of largest subordinate follicle (0.94±0.09 vs 0.62±0.1 mm/day) between Day 1 to 3 of the examination. The mean (±SEM) number of follicles with respect to wave emergence in different size categories, viz. <4 mm (i.e. between 2 mm to <4 mm), 4–6 mm and >6 mm are shown in Fig. 2 (lower panel). The number of <4 mm follicle started to decrease (P<0.05) on next day of wave emergence (Day 2 of ablation) while the number of follicles of 4–6 mm remained constant during the examination period (Day 0 to 4 of ablation). Subsequently, all buffaloes in group 1 (15/15; 100%) exhibited estrus within 48–72 h of CIDR removal and PGF administration. Further 86.6% of group 1 buffaloes (13/15) ovulated within 24-72 h of estrus (n=1 at 24 h, n=3 at 36 h, n=6 at 48 h, n=1 at 60 h and n=2 at 72 h). The interval from CIDR removal to ovulation was 81.2±3.08 h (n=7 at 72 h, n=2 at 84 h and n=4 at 96 h). Out of the two anovulatory buffaloes, one had follicular cyst development while the second revealed persistent follicle with partial luteinization of the follicular wall. In group 2, only 2 buffaloes exhibited estrus (22.2%). The first service pregnancy rate in group 1 was higher (P<0.05) than that in contemporary control group (10/15, 66.67% vs 2/9, 22.2%). The size of CL at Day 5 post-ovulation in group 1 was larger (P<0.05) in pregnant buffaloes (14.7±0.65 mm, n=10) than the CL in non-pregnant ones (10.6±1.5 mm, n=3; those otherwise ovulated).

In the present study, ultrasound-guided transvaginal aspiration of follicles <5 mm, regardless of stage of the estrous cycle, resulted in the synchronous emergence of a new wave within 1–2 days in all anestrous buffaloes which is analogous to previous observations in cattle, other bovids and cervids (Palomino *et al*. 2014, Honparkhe *et al*. 2014). Following CIDR removal and PGF administration, all buffaloes from the group 1 exhibited estrus within 48–72 h and majority (86.6%) ovulated within 9–11 days from follicular wave emergence. Similar results were observed in ablation-induced follicular wave in cattle (Mapletoft *et al*. 2003, Amiridis *et al*. 2006). The maximum diameter of preovulatory follicle was numerically smaller (11.3±0.52 mm) as compared to that observed in previous study (12–15 mm) that used progesterone device in anestrous buffaloes (Singh *et al*. 2009b). The buffaloes that became pregnant had bigger preovulatory follicle (12.1±0.6 vs 9.7±0.7; P<0.05) and bigger CL (14.7±0.65 mm versus 10.6±1.5 mm) after ovulation compared to the non-pregnant buffaloes. Our findings are in agreement with previous observations that a bigger preovulatory follicle leads to bigger and more functional CL (Dadarwal *et al*. 2013). It is well documented that the establishment and maintenance of pregnancy is greatly dependent on the ability of CL to produce progesterone (Goff 2002).

Water buffaloes show seasonality in reproductive pattern and undergo a period of low sexual activity in summer months, i.e. June –September (Barile 2005). In Northern India (the location of the present study), delayed resumption of post-partum ovarian activity is observed during June-September as compared to that in other months in buffaloes (Aksoy *et al.* 2002). Various factors, viz. environment, nutrition and management resulting in hormonal asynchrony are considered responsible for these conditions (Das and Khan 2010). At the start of our study (October) buffaloes had just crossed the summer and that could be the reason for their reproductive quiescence in the previous 3 to 4 month. Hormonal treatments (including single/double PGF, Ovsynch) have been used to achieve optimum reproductive efficiency throughout the year. However, conception rates following these regimens are reduced during changeover from the breeding to non-breeding season (Baruselli 2001, Neglia *et al*. 2003).

The combination of synchronization of wave emergence and ovulation and proper progesterone environment during dominant follicle development (follicular ablation + CIDR + GnRH) used in our study gave appreciably high pregnancy rates (66.67%) as compared to untreated ones and the previously reported protocols (Paul and Prakash 2005, Presicce *et al*. 2005, Murugavel *et al*. 2009). Although timing of fixed-time AI did not rely on estrus detection; nevertheless, majority of buffaloes (86%) exhibited signs of estrus. Perhaps, the combination of a growing follicle with concomitant changes in progesterone plasma levels (as a result of exogenous progesterone manipulations) were responsible for proper estrus behaviour. It is noteworthy that timing from CIDR removal to ovulation and estrus expression to ovulation were equally consistent (i.e. had almost similar S.E.M values); therefore, the former could be used without the need to detect estrus.

In conclusion, our results supported the hypothesis that the combination of follicular ablation and intravaginal progesterone device is efficacious for synchronizing estrus and ovulation for fixed-time AI to improve pregnancy rates in anestrous buffaloes.

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