



Follicular wave emergence and fertility following estrogen based synchronization regimen in anestrus buffaloes (*Bubalus bubalis*)

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Received: 1 October 2013; Accepted: 12 December 2013

Keywords: Buffalo, CIDR, Estradiol-17 β , Wave emergence

Applying estrus synchronization regimens in buffaloes increases the production period (Ghuman *et al.* 2008). Estradiol in combination with progesterone administered at the beginning of a CIDR-treatment results in synchronous emergence of a new follicular wave in both heifers and pluriparous bovines (Martinez *et al.* 2000). Estradiol is associated with uterine-induced luteolysis, however in combination with progesterone causes follicle regression followed by synchronous emergence of a new follicular wave (Martinez *et al.* 2005). In buffaloes, the effects of estradiol 17 β (E17 β), Estradiol Benzoate (EB) or Estradiol Valerate (EV) on WE have not been critically studied, however, a study in buffaloes shows that the use of lower doses of E17 β (1.5 mg, i.m.) leads to synchronous emergence of follicular waves (Honparkhe 2012). The present study was designed with the objective to investigate the effect of exogenous estradiol (E17 β) + Controlled Internal Drug Release Device (CIDR) on follicular wave emergence and subsequent ovulation and conception rate in anestrus buffaloes.

The study was conducted on 30 Murrah buffaloes with the history of anestrus. Buffaloes were administered CIDR device (1.38 g P4) and concurrently received 1.5 mg estradiol-17 β in 1.5 ml canola oil (i.m.). CIDR removal and a prostaglandin administration F₂ α (PGF) on day 9 were followed by injection of Gonadotropin Releasing Hormone (GnRH) analogue (20 μ g, i.m.) 48 hours later. Buffaloes were inseminated twice at 12 h and 24 h following GnRH injection. Control group received same injections except the estradiol-17 β at the time of CIDR insert (Fig.1). Trans-rectal ultrasonographic examinations were made by a single operator with a B mode ultrasound scanner, equipped with inter-changeable 5/7.5 MHz linear array rectal transducer.

The fate of largest follicle present at the time of treatment (E2 + CIDR) was recorded till 4–5 days/up to the emergence of new wave. The results presented in table, show that the mean diameter of largest follicle was reduced from 8.14

(0th day) to 5.42 mm on the day of wave emergence. In control, the size was reduced over a prolonged duration of 6 days. The mean number of 4–5 mm follicles increased from 1.58 (0th day) to 5.75 follicles in Group I and 1.52 to 4.88 follicles in control group on day 4. This indicates a sharp decrease in largest follicle size and more increase in emergence follicles in Group I, suggesting a better synchrony in estrogen treated animals.

The logic behind the use of estradiol-17 β in the present study in buffalo for synchronization of wave emergence is that it has shorter half-life and requires lesser doses compared to other esters of estrogen. Buffalo are sensitive to exogenous estradiol as there is decrease in milk production but the use of E-17 β (@1.5 mg) has no effect on milk production (Honparkhe 2010). The smaller doses of E-17 β (1.5 mg) used in the present study was found equally effective to the larger doses (2.5 mg, 5 mg and 10 mg) used in cattle to suppress the dominant follicle (Siqueira *et al.* 2009 and Honparkhe *et al.* 2010).

The constant reduction in the mean diameter of largest follicle till the day of wave emergence and increase in number of 4–5 mm follicles in the ovary in the present study indicates new wave emergence earlier in estrogen based group than control. The studies regarding the new wave emergence following exogenous hormone treatments have been widely reported in cattle (Siqueira *et al.* 2009). Estradiol is incorporated into progesterone-based estrus synchronization regimens to cause uterine-induced luteolysis; however, recent studies in cattle have demonstrated the administration of estradiol in combination with progesterone causes follicle regression followed by synchronous emergence of a new follicular wave (Martínez *et al.* 2005). The consistent regression of the largest follicle observed until a new wave in present study is favoured by

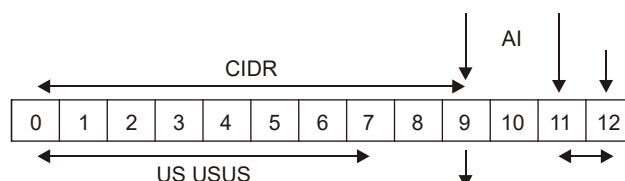


Fig.1.Schematic diagram of control (US=ultrasnography).

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Table 1. Daily ultrasonographic measures of follicular size up to wave emergence in group I and II

	Day prior to WE	0 D	1 D	2 D	3 D	4 D	6 D
Group IE-17 β +CIDR	Size of LF (mm)	8.14 $\pm$ 0.63 ^a	5.45 $\pm$ 0.62 ^b	5.00 $\pm$ 0.33	5.49 $\pm$ 0.32	5.42 $\pm$ 0.15	–
	No. of 4–5 mm follicles	1.58 $\pm$ 0.23 ^a	1.85 $\pm$ 0.18	2.50 $\pm$ 0.23	5.00 $\pm$ 0.22	5.75 $\pm$ 0.21 ^b	–
Group IICIDR (control)	Size of LF (mm)	8.55 $\pm$ 0.63 ^a	7.21 $\pm$ 0.62	6.33 $\pm$ 0.33	6.41 $\pm$ 0.42	6.19 $\pm$ 0.32	5.11 $\pm$ 0.31 ^b
	No. of 4–5 mm follicles	1.52 $\pm$ 0.21 ^a	1.75 $\pm$ 0.12	2.80 $\pm$ 0.23	4.82 $\pm$ 0.22	4.88 $\pm$ 0.42	5.22 $\pm$ 0.31 ^b

WE, Wave emergence; LF, Largest follicle; D, days; ^a vs ^b, P<0.05 between the row.

previous reports in cattle where consistent regression of dominant follicles and synchronization of new follicular wave was achieved by using 5 mg EB in association with 100 mg progesterone. Also, estradiol administration has also been observed to cause growth of dominant follicle, leading to plateau phase followed by its regression and new wave emergence (Siqueira *et al.* 2009). In the present study estradiol might have acted to generate FSH surge to increase the number of smaller follicles resulting in emergence of new wave, which is indicated by reports available to favour the increase in 4–5 mm follicles around the wave emergence due to the increase in FSH and recruitment of the cohort of ovarian follicles (Wiltbank *et al.* 2002). Following emergence, follicles continue to grow but FSH begins to decline until the time of follicular deviation. Moreover the increased number of 4–5 mm follicles was also attributed to suppression of gonadotropin followed by regression of existing dominant follicle and emergence of new follicular wave (Mapletoft *et al.* 2003 and Siqueira *et al.* 2009).

Further in the present study, the size of dominant follicle observed in estradiol group was higher than control (13.20 vs 12.00 P<0.05). Also, the percentage of animals ovulated was around 85% vs 60% with acceptable first service conception rate 47 vs 30%. The higher ovulation rates might be as a result of larger diameter of ovulatory follicles achieved in the present study. The literature for such comparison in buffalo is sparse. However, Martinez *et al.* (2005) have studied the effects of estradiol on ovarian follicular dynamics in CIDR-treated beef cattle and also achieved good conception rates. The estradiol-17 β concurrent with CIDR results in early emergence of new wave which might allow the follicle more time for its growth and maturation to the ovulatory stage and hence improved ovulation and conception rates in buffalo.

SUMMARY

The study was undertaken to study the follicular changes around the time of new wave emergence following estradiol-17 β (E-17 β) + controlled internal drug release (CIDR) based synchronization in 30 anestrus buffaloes. The animals were divided in two Groups, viz. Group I and II. In Group I (n=20), on day 0 (beginning of experiment), buffaloes were administered CIDR device (1.38 g P4) and concurrently received 1.5 mg estradiol-17 β in 1.5 ml canola oil (i. m.). On day 9, CIDR was removed and a prostaglandin

(PG) F₂ α analogue (500 μ g, i. m.) was administered. On day 11, buffaloes were administered gonadotropin releasing hormone (GnRH) analogue (20 μ g, i. m.) and inseminated twice at 12 h and 24 h following GnRH injection. Group II (control, n=10) received the CIDR-based protocol except the administration of estradiol at the time of CIDR insert. Ovaries were examined for follicular wave emergence, ovulation of dominant follicles at estrus and subsequent conception at 45 days of insemination. The mean diameter of largest follicle was reduced from 8 mm (0th day) to 4 mm on the day of wave emergence (WE), i.e. day 4 in Group I but day 6 in control. The mean number of 4–5 mm follicle was 5.7 in Group I and 4.8 in control on the days of WE. Ovulation and conception rates achieved were 85 vs 60% and 47 vs 30% respectively. In conclusion the new wave emergence was induced earlier by estradiol-17 β administered along with CIDR which ultimately resulted in more ovulation and conception rate in buffalo. Estrogen based protocols might be adopted to improve fertility in anestrus buffaloes.

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