Comparative efficacy of E-17β and GnRH administration on day 0 of a controlled internal drug release (CIDR) based protocol on synchrony of wave emergence, ovulation and conception rates in Murrah buffalos (*Bubalus bubalis*)

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Summary

The study was undertaken to compare the efficacy of an estradiol-17β + CIDR based protocol with the conventional ovsynch + CIDR based protocol for synchrony of wave emergence and ovulation in Murrah buffalos. In group I (n=25), on day 0 (beginning of experiment), buffaloes were administered a CIDR device (1.38 g P_4) and concurrently received 1.5 mg E-17β. On day 9, the CIDR was removed and a prostaglandin (PG) $F_2\alpha$ analogue (500 µg) was administered. On day 11, buffaloes were administered a gonadotropin releasing hormone (GnRH) analogue (20 µg) and inseminated twice at 12 h and 24 h following GnRH injections. Group II (n=25) protocol was based on an ovsynch regimen plus CIDR for 7 days followed by double insemination at induced estrus. Group III (n=10) served as control and was not given any hormone on day 0 of the protocol. In groups I, II and III, the duration of new follicular wave emergences were observed on days 4.22 ± 0.12, 3.12 ± 0.33 and 5.14 ± 0.42, respectively. In group I, synchrony of wave emergence was more and the diameter of pre-ovulatory follicles was larger ($P<0.05$) compared to groups II and III. The first service conception rate (FSCR) was higher ($P<0.05$) in group I while ovulation rates were not different between groups I and II. In conclusion, more synchrony of wave emergence, larger diameter of dominant follicles and higher first service conception rate was observed following the E-17β + CIDR based protocol in buffalos.

Key words: Buffalo, Conception rate, Estradiol, Ovulation, Wave emergence

Introduction

Applying estrus synchronization regimens may provide a potential alternative, but to date, partial success has been achieved in improving the reproduction potential of buffalos (Ghuman *et al*., 2010). Originally, estradiol was incorporated into progesterone-based estrus synchronization regimens to cause uterine-induced luteolysis. However, recent cattle studies have demonstrated that the administration of estradiol in combination with progesterone causes follicle regression followed by the synchronous emergence of a new follicular wave (Martinez *et al*., 2005). The synchronous emergence of a follicular wave prior to estrus synchronization protocols provides ovulation synchrony that allows fixed-time AI without the need to detect estrus (Pursley *et al*., 1995). In ovsynch regimens, the first GnRH usually increases plasma progesterone by inducing the ovulation of a dominant follicle, thereby synchronizing the emergence of a new follicular wave. The second-GnRH synchronizes the pre-ovulatory luteinizing hormone (LH) surge and ovulation (Martinez *et al*., 1999). It has recently emerged that more precise manipulations of follicular developments may be needed to achieve better synchrony of ovulation and fertility. For synchronized follicular wave emergence, approaches such as trans-vaginal ultrasound guided follicular ablation or estradiol have been used instead of GnRH (Siqueira *et al*., 2009). Out of the various esters of estradiol, treatment with formulations of estradiol benzoate (EB), estradiol valerate (EV) or estradiol cypionate (ECP) can induce new follicular waves at inconsistent and prolonged intervals due to their long half-life compared to estradiol-17β (E-17β) (Vynkier *et al*., 1990; Bo *et al*., 1994). In buffaloes, contemporaneous comparisons of the effects of E-17β, EB or EV on gonadotropin concentrations in estrus synchronization protocols have not been critically studied; however, in a recent study, the use of lower doses of E-17β (1.5 mg, i.m.) were found to lead to the synchronous emergence of follicular waves (Honparkhe, 2012). The interval between the treatment to the emergence of a new follicular wave has been suggested to influence pregnancy rates, possibly because of the growth and size of new pre-ovulatory follicles (Kim *et al*., 2005). Moreover, according to previous reports, in a progesterone based protocol, even the time of emergence of a new follicular wave may be important for high pregnancy rates (Moreno *et al*., 2001; Utt *et al*., 2003). There is a scarcity of studies regarding the use of E-17β based protocols in buffalos. In addition, the impact of inducing new follicular wave emergence on subsequent

pregnancies remains to be studied in this species. The present study was, therefore, designed with the objective of investigating the effect of exogenous estradiol (E-17β) in a CIDR based protocol on the duration of follicular wave emergence, ovulation and conception rates in buffaloes.

Materials and Methods

The study was conducted on 60 Murrah buffaloes from a private dairy farm in Ludhiana, a centrally located district in Punjab (between north latitude 30°-34' and 31°-01', east longitude 75°-18' and 76°-20' and an altitude of 180-300 meters above sea level). The place is characterized by dryness, except for a brief spell at the monsoon season, a very hot summer and a bracing winter. Buffaloes were fed 10-15 kg green fodder, 8-10 kg wheat straw and 2-3 kg concentrate daily, and had *ad libitum* access to drinking water and wallowing in a pond of water. Animals selected were between their second to fourth parity. Their body weight ranged between 400-500 kg and their body condition score was 4-4.5 as per the guidelines of the BCS chart. The buffaloes were assigned a score from 0 (emaciated) to 5 (obese) as per Vanessa *et al*. (2009) for African buffalo. All animals were milked twice daily and housed under a semi-loose housing system.

Treatments

The animals were treated randomly following the protocols as group I, II and III.

Group I, Estradiol + CIDR based protocol (n=25, Fig. 1)

The animals were administered an intra-vaginal CIDR device (Pfizer India Ltd.) and 1.5 mg of E-17β (sigma laboratories, i.m.) on day 0 of the protocol. On day 9, the CIDR was removed and the $PGF_{2\alpha}$ analogue was given (Cloprostenol sodium, 500 µg, i.m., Vetmate, Vetcare, Provimi, India). On day 11, buffaloes were administered a GnRH analogue (Buserelin acetate, 20 µg, i.m., Receptal, Intervet, India). All the buffaloes were inseminated on day 11 (12 h) and day 12 (24 h).

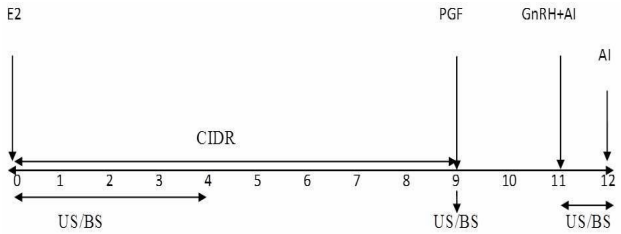

Fig. 1: Schemetic diagram of estrodiol-based protocol (group-I, n=25). E_2: Estradiol-17β, PGF: Prostaglandin$F_{2\alpha}$, GnRH: Gonadotrpin releasing hormone, US: Ultrasonography, and BS: Blood sample

Group II, Ovsynch + CIDR-based protocol (n=25, Fig. 2)

Instead of E-17β, a GnRH analogue was administered concurrently with the CIDR. On day 7, the CIDR was removed and each buffalo was administered (i.m.) a $PGF_{2\alpha}$ analogue. On day 9, buffaloes were administered the same GnRH analogue again (20 µg, i.m.). All the buffaloes were inseminated on day 9 (12 h) and day 10 (24 h).

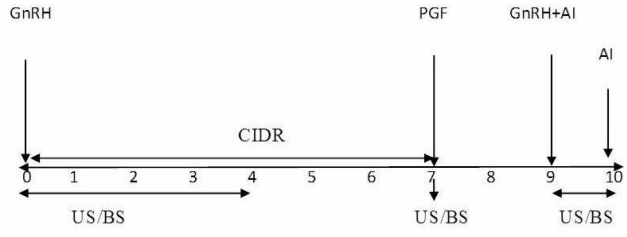

Fig. 2: Schematic diagram of ovsynch-based protocol (group-II, n=25). GnRH: Gonadotrpin releasing hormone, PGF: $PGF_{2\alpha}$, US: Ultrasonography, and BS: Blood sample

Group III, CIDR-based protocol (no hormone on day 0, n=10, Fig. 3)

The animals were only administered the CIDR device on day 0. The rest of the protocol and insemination procedures were same as group II.

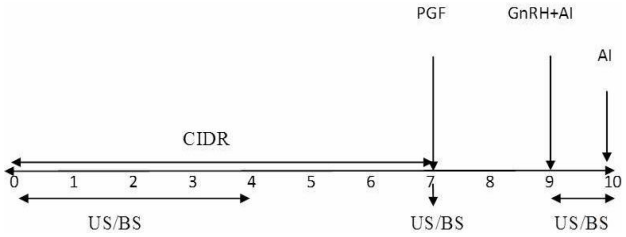

Fig. 3: Schematic diagram of control group (group-III, n=10)

Ultrasonography

Trans-rectal ultrasonogaphic examinations were made by a single operator with a B mode ultrasound scanner (Agroscan, AL, ECM, Angouleme, France) equipped with an inter-changable 5/7.5 MHz linear array rectal transducer (ALR, 575 probe, ECM, Angouleme, France). The ovaries (ovarian follicles) were monitored on days 10, 0 (beginning of the experiment), 1-4, 7, 9, 11, and 12. Ovaries were examined for follicular wave emergence (presence of multiple 4 mm follicles), antral diameter of largest follicle(s)/visible CL and occurrence of ovulation.

Plasma progesterone analysis

Plasma progesterone was assayed for wave emergence and ovulation days in all the groups (the days were decided by ultrasonography), with a solid-phase radioimmunoassay using antisera raised in our laboratory (Ghuman *et al*., 2009). Sensitivity of the assay was 0.1 ng/ml, intra- and inter-assay variation coefficients were 6.2% and 9.5%, respectively.

Plasma estradiol analysis

Plasma E-17β (E_2) was estimated for wave emergence and ovulation days, using a commercially

available Direct Immuno-Enzymatic Assay kit (Monobind Inc., USA, Accubind estradiol ELISA microwells), using Anti-estradiol IgG coated wells. Absorbance of each well was taken at 450 nm within 30 min. The standard curve was elaborated with the 4-parameter curve fitting system and the estradiol concentration in the samples was calculated.

Conception rate

The first conception rate between the groups was noted by diagnosing pregnancy through ultrasonography on day 60 and rectal palpation on day 90 post-AI.

Statistical analysis

A Student's t-test and ANOVA were run to compare parameters between the groups. First service conception rate was compared by Chi-square analysis.

Results

The proportion of animals showing wave emergence until five days from the beginning of treatment in groups I, II and III was 84 (21/25), 76 (19/25) and 40 (4/10) percent, respectively ($P<0.05$). Follicular wave emergence was observed on days 4.22 ± 0.12, 3.12 ± 0.33 and 5.14 ± 0.42, respectively, following CIDR insertion in groups I, II and III (Table 1, $P<0.05$). As shown in Table 2 for group I, II and III animals, the mean diameter of the largest follicle was reduced from about 8 (day 0) to 5 mm around the day of WE. Also, the mean number of 4-5 mm follicles increased from 1.58 (day 0) to 5 around the day of WE ($P<0.05$). The proportion of buffaloes with follicular wave emergence within 5 days after treatment was 84% (21/25), 64% (16/25) and 40% (4/10), for group I, II and III respectively ($P<0.05$). The proportion of buffaloes with follicular wave emergence within 5 days after treatment was higher in group I and II compared to the control group ($P<0.05$). Also, the proportion was higher in group I compared to group II ($P<0.05$). Data presented in Table 3 shows that in all groups, mean levels of plasma progesterone (P_4) on the days of wave emergence were higher compared to levels on the day of ovulation. The means of P_4 and E_2 levels on the day of ovulation were not different between the groups ($P>0.05$). The size of the pre-ovulatory follicle obtained in group I was larger than group II; however, no statistical difference was observed between either of these groups and the control (14.6, 13.2 and 11.4 mm as shown in Table 1). The percentage of animals which ovulated in the present study was 92 (23/25) for group I, 84 (21/25) for group II and 70% (7/10) for the control group. First service conception rates were 48, 37 and 31%, for groups I, II and the control, respectively ($P<0.05$ for group I and the control).

Discussion

The present study aimed to investigate the effects of E-17β or GnRH in a progesterone based protocol on day 0 of treatment, on the duration of new wave emergence, endocrine changes, and subsequent ovulation in buffalos.

Table 1: Means (±SEM) of day of wave emergence (WE), day of ovulation and size of dominant follicle (DF) in estradiol-based vs ovsynch-based regimens

Parameter	Estradiol+CIDR-based, (n=25)	Ovsynch+CIDR based, (n=25)	CIDR-based control (n=10)
WE (days)	4.2 ± 0.15^{a}	3.8 ± 0.36^{b}	5.1 ± 0.33^{1}
DF size(mm)	14.62 ± 0.38^{c}	13.2 ± 0.2	11.4 ± 0.38^{2}

^{a} vs ^{b} and ^{1}, ^{c} vs ^{2} $P<0.05$ between the rows

Table 2: Ultrasonic measures of follicles up to wave emergence in groups I and II

Group I (n=25)						
Days prior to WE	0 D	1 D	2 D	3 D	4 D	5 D
Mean size of LF (mm)	8.14 ± 0.63^{a}	5.45 ± 0.62^{b}	5.00 ± 0.33	5.49 ± 0.32	5.49 ± 0.32	5.49 ± 0.32
Mean No. of 4-5 mm F	1.58 ± 0.23	1.85 ± 0.18	2.50 ± 0.23^{c}	5.00 ± 0.22^{d}	5.75 ± 0.23	5.81 ± 0.33
Group II (n=25)						
Mean size of LF (mm)	8.25 ± 0.63^{A}	5.11 ± 0.65^{B}	5.12 ± 0.42	5.34 ± 0.51	5.10 ± 0.32	
Mean No. of 4-5 mm F	1.29 ± 0.52	1.95 ± 0.66	2.82 ± 0.35^{C}	5.23 ± 0.42^{D}	5.86 ± 0.55	
Group III (n=8)						
Mean size of LF (mm)	9.15 ± 0.23	7.31 ± 0.55	6.32 ± 0.42^{1}	5.54 ± 0.51	4.50 ± 0.32^{2}	
Mean No. of 4-5 mm F	2.18 ± 0.43	2.55 ± 0.38	2.90 ± 0.33^{3}	5.50 ± 0.32^{4}	5.66 ± 0.13	

^{a} vs ^{b}, ^{c} vs ^{d}, ^{A} vs ^{B}, ^{C} vs ^{D}, ^{1} vs ^{2} and ^{3} vs ^{4} $P<0.05$ between the rows (WE: Wave emergence, LF: Largest follicle, and F: Follicle)

Table 3: Plasma progesterone (P_4) and estradiol (E_2) levels on wave emergence and ovulation days

Day of cycle	Group I		Group II		Group III	
Day	P_4 (ng/ml)	E_2 (pg/ml), (n=14)	P_4 (ng/ml)	E_2 (pg/ml), (n=14)	P_4 (ng/ml)	E_2 (pg/ml), (n=10)
Day of WE	1.18 ± 0.14^{a}	0.30 ± 0.70^{c}	1.65 ± 0.22^{a}	0.52 ± 0.20	1.03 ± 0.34^{a}	0.55 ± 0.20
Day of ovulation	0.62 ± 0.07^{b}	0.84 ± 0.33^{d}	0.52 ± 0.05^{b}	0.70 ± 0.30	0.72 ± 0.25^{b}	0.84 ± 0.33

^{a} vs ^{b} and ^{c} vs ^{d} $P<0.05$ between the columns

In the present study, the proportion of animals showing follicular wave emergence was higher in group I compared to group II. This shows that in the present study, the use of lower doses of E-17β along with a CIDR led to more synchrony of emergence of follicular waves in Murrah buffaloes. Similar observations were documented in cattle (Caccia and Bo, 1998; Martinez *et al*., 2005) and buffalo (Niasari-Naslagi *et al*., 2007; Honparkhe, 2012), where the administration of estradiol in combination with progesterone resulted in better synchrony of a new follicular wave emergence. The proportion of buffaloes with follicular wave emergence was higher in group I and II compared to the control group ($P<0.05$). These findings are different from those of Kim *et al*. (2005) who reported that following estradiol and GnRH treatments, the proportion of cows with follicular wave emergence within 7 days of the treatment was higher in GnRH than EB. Thus, E-17β at lower doses may be equally or even more effective as higher doses of estradiol esters in cattle. In addition, the administration of either estradiol or GnRH on the day of CIDR insertion can aid the emergence of a new follicular wave in buffalos.

The mean interval from treatment to follicular wave emergence (3.8 days) in the GnRH group in the present study was slightly longer than that reported for cattle by Kim *et al*. (2005) (2.9 days) and Pursley *et al*. (1995) (2.1 days). This could be related to the species' differences in treatment responses. The mean interval to follicular wave emergence in the E-17β + CIDR group was similar to previous reports for cattle by Bo *et al*. (1996) and Caccia and Bo (1998), in which follicular wave emergence occurred 5.4 and 4.0 days (with a range of 3 days) after treatment of cattle with 5 mg of EB. The administration of E-17β in the present study resulted in the emergence of a new follicular wave to be later compared to GnRH. The longer duration of wave emergence following E-17β administration in the present study is favored by a CIDR-based study in cattle by Martinez *et al*. (2005), where mean intervals (ranges) to follicular wave emergence in E-17β and GnRH groups for beef heifers were 3.4 days and 1.5 days. Also, the mean interval from estradiol treatment to follicular wave emergence in the present study is consistent with previous reports for cattle (Martinez *et al*., 2002; Colazo *et al*., 2004; Kim *et al*., 2005) and buffaloes (Niasari-Naslagi *et al*., 2007), where a new follicular wave occurred around the 4th day of treatment. Follicular wave emergence in progestagen-treated cattle has been reported to occur 4.3 days after the administration of 5 mg of E-17β and 5.4 days after the administration of 5 mg of EB (Caccia and Bo, 1998; Colazo *et al*., 2004). Bo *et al*. (1994) incorporated progesterone with the initial treatment of estradiol for follicular wave synchronization to prevent an estrogen-induced LH surge.

Previous reports revealed that in an estradiol-based protocol, the mechanism involved in follicular wave emergence is suppression of follicle stimulating hormone (FSH) and LH in a progesterone-high environment, thus leading to the regression of FSH and LH dependent follicles. Once regression begins and exogenously administered estradiol is metabolized, FSH surge is generated and a new follicular wave emerges about 4 days later in cattle (Siqueira *et al*., 2009). This high progesterone environment was provided by inserting a CIDR at the time of the exogenous administration of E-17β in the present study. Accordingly, the emergence of a new wave could be due to the inhibition of an LH surge following the treatment (the effects on LH and FSH levels could not be observed in the present study).

In the present study, there was a continuous decrease in the size of the largest follicle observed on the day of treatment followed by a decrease in estradiol levels until a new wave occurred. The fate of the largest follicle present at the time of treatment (E_2 + CIDR) was recorded until 4-5 days or up to the emergence of a new wave. The new wave was characterized by a decrease in the diameter of the largest follicle and an increase in the number of 4-5 mm follicles in the ovary. Estradiol-17β has been shown to cause uterine-induced luteolysis; however, recent studies in cattle have demonstrated that the administration of estradiol in combination with progesterone causes follicle regression followed by a synchronous emergence of a new follicular wave (Martinez *et al*., 2005). Thus, it can be concluded from the present study as well as previous reports available for cattle, that in buffaloes, the use of estradiol along with progestin supplements might affect the synchronization of follicular waves.

In the present study, there was an increase in the number of 4-5 mm follicles around the day of emergence, which is well supported by studies of cattle (Colazo *et al*., 2004) and buffalos (Honparkhe, 2012) reporting on increases in the number of smaller follicles at wave emergence. Studies suggest that the increase in the number of smaller follicles could be due to the increase in FSH and recruitment of the cohort of ovarian follicles and wave emergence (Adams *et al*., 1992). Furthermore, the increased number of 4-5 mm follicles has also been attributed to the suppression of gonadotrophin followed by the regression of existing dominant follicles and the emergence of a new follicular wave (Mapletoft *et al*., 2003). A similar hypothesis could be applied to the increased number of 4-5 mm follicles in the present study.

In the present study, estradiol levels decreased after estradiol administration while mean plasma progesterone levels were not different before and after the administration. Bo *et al*. (1994) have also observed that after treatment with 5 mg E-17β, peripheral estradiol concentrations in cattle returned to a baseline within 48 h of injection. In the present study, plasma estradiol concentrations decreased within 24 h of treatment, which could be attributed to the higher sensitivity of buffaloes to E-17β. Siqueira *et al*. (2009) have also reported the growth of a dominant follicle, leading to a plateau phase followed by regression, and a new wave emergence following estradiol administration in cattle. On the day of ovulation, mean P_4 and E_2 levels were not different between groups I and II ($P>0.05$). Furthermore, in a few

animals of the estradiol-based group (n=12, data not presented), plasma samples were collected 72 h post-CIDR removal when progesterone levels were found to reach their lowest (0.33 ng/ml) and estradiol levels further increased. These lowest P_4 levels probably coincided with the period around the time of ovulation (observed by ultrasonography). It was recently observed in buffalos that response to a luteolytic dose of $PGF_{2\alpha}$ administered on the day of CIDR removal was good ($P<0.05$) and there was decline in plasma progesterone during the subsequent period of 72 h (Ghuman *et al*., 2012). Ghuman *et al*. (2010) also suggested a decline of plasma progesterone to basal concentrations within 2-3 days after $PGF_{2\alpha}$ injection and CIDR withdrawal. This was also the case in the present study, where we observed a decline at 48-72 h after CIDR withdrawal, the period coinciding with estrus. It could be thus be concluded that FTAI must be carried out at least 60 h (time of ovulation) after CIDR removal.

Differences in the size of pre-ovulatory follicles were presumed to be caused by the regression of spontaneous corpora lutea, present on the ovary with E-17β, which might help in the stimulation of follicular growth. Moreover, the larger size of pre-ovulatory follicles in group I compared to group II might be attributed to the two additional days of CIDR exposure, which have already been suggested to increase follicle growth rate (Atkins *et al*., 2010). Similar suggestions have also been provided by Pandey *et al*. (2010) regarding larger sized follicles in buffalos being responsible for higher plasma concentrations of estradiol.

The optimal ovulation rate achieved with the combination of E-17β and CIDR in the present study is supported by another work on beef heifers (Caccia and Bo, 1998), where during an estrus synchronization protocol, the combination of E-17β and intra-vaginal progesterone resulted in the ovulation of 75% of the heifers between 72 and 84 h following device removal and PGF administration. In the present study, the larger diameter of dominant follicles as well as higher first service conception rates indicate the superiority of an estradiol-based protocol over traditional ovsynch-based synchronization regimens, thereby supporting the use of such synchronization protocols for buffalos. Further achievement of higher first service conception rates in estradiol-based protocols in buffalos could be attributed to the larger diameter of dominant follicles and higher plasma estradiol levels which might be responsible for timed ovulation and the improvement of conception chances following timed AI in buffalos.

In conclusion, although the replacement of first GnRH with E-17β in an ovsynch plus CIDR protocol resulted in longer wave emergence duration, it lead to a better wave emergence synchrony, a larger diameter of the dominant follicle and higher first service conception rates in buffalos. Thus, the achievement of a better wave emergence synchrony, dominant follicles' larger diameter and higher first service conception rates may be expected following a E-17β + CIDR based protocol in buffalos.

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