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Fertility Response Following Estradoublesynch and Progesterone Based Ovsynch Protocols in Delayed Pubertal Buffalo Heifers

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ABSTRACT

The study evaluated the fertility response following Estradoublesynch and progesterone-based Ovsynch protocols in delayed pubertal buffalo heifers. A total of twenty delayed pubertal buffalo heifers were subdivided in two equal subgroups. Group I animals were treated with PGF2α analogue (500 µg cloprostenol sodium) i.m. on day 0, GnRH analogue (20 µg buserelin acetate) on day 2, PGF2α analogue (500 µg cloprostenol sodium) repeated on day 9 and estradiol benzoate 1 mg intramuscular (i.m.) on day 10. Fixed-time artificial insemination (FTAI) was done at 48 and 60h. Group II animals were subjected to GnRH analogue (20 µg buserelin acetate) i.m. along with CIDR insert intravaginally on day 0, PGF2α analogue (500 µg cloprostenol sodium) i.m. and removal of CIDR on day 7, GnRH analogue on day 9 followed by FTAI at 12 and 24h. Blood collection (plasma P4) and ultrasonography (luteal and follicular dynamics) was performed on day 0, 9, 10, 12 in group I and day 0, 7, 9 and 10 in group II animals. Pregnancy diagnosis was made at 45-60 days post AI. Estrus response was observed in all the buffalo heifers of group I and II. First service conception rate was significantly higher in group I (50%) compared to group II (20%) ($P<0.05$). Overall pregnancy rate was almost similar in both group I and II (60% vs 70%). In conclusion, estradoublesynch and progesterone-based ovsynch protocol can effectively induce ovulatory estrus and improve pregnancy rates in delayed pubertal buffalo heifers.

Key words: Delayed Puberty, Buffalo, Heifers, Estradoublesynch, CIDR, Ovsynch.

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INTRODUCTION

Puberty is a process of gaining reproductive competence. In heifers, puberty can be characterized in several ways *viz.* age at first estrus (heat), age at first ovulation and/ or age at which a female can support pregnancy without difficulty (Senger 1999). In buffalo, puberty is comparatively attained at a later age (2.5-3 years) than in cattle (1.5-2 yrs).

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Under the favourable conditions, the riverine buffaloes exhibit their first estrus at 15-18 months of age, whereas the swamp buffaloes do so at 21–24 months (Borghese and Mazzi 2005). The onset of puberty is the function of complex and integrated cascade of biological events leading to progressive maturation of sexual characteristics, especially overt estrus, that ultimately leads to the attainment of sexual maturity i.e. full reproductive capacity. Delayed attainment of puberty is one of the major reproductive problems in buffaloes. The incidence of delayed puberty in buffalo was reported as 33% in the farms maintained by marginal and sub marginal farmers (Honparkhe *et al.,* 2019) which is significantly lower than the incidence reported in pubertal anestrus buffaloes (56%) (Baitule *et al.,* 2016). Earlier it was observed that delayed pubertal animal show certain variation in plasma biochemical profile which may be attributed to the delay in puberty (Pothireddy *et al*., 2020; Ahuja and Parmar, 2017). Even though the animal achieves the desired chronological age as well as body weight but the estrus does not occur. This delay in puberty is succeeded by corresponding delay in sexual maturity, conception and age at first calving, leading to the low reproductive efficiency in buffaloes and lengthening their non-productive life.

Different hormonal and non-hormonal methods have been tried to augment fertility in delayed pubertal buffalo heifers but with variable success rates (Naidu *et al.,* 2009; Baitule *et al.,* 2016). Estradoublesynch (Mirmahmoudi *et al.,* 2014a) and progesterone based ovsynch protocols (Ghuman *et al.,* 2015) have been used in post-partum anestrus and subestrous buffaloes; but the similar studies using these hormonal protocols are lacking in delayed pubertal buffalo heifers. Hence the present study was conducted to assess estrus induction and conception rates following estradoublesynch and progesterone based ovsynch protocols in delayed pubertal buffalo heifers.

MATERIALS AND METHODS

The study was conducted on 20 apparently healthy delayed pubertal Murrah buffalo heifers reared at private dairy farms without any history of estrus exhibition and functional corpus luteum despite having age ≥ 3 years, body weight ≥ 250 kg and body condition score (BCS) of 2.5-3 on 5-point scale. Animals were randomly divided into groups with ten heifers in each group *viz.* Group I (Estradoublesynch; 'EDS' group) and Group II (CIDR+ Ovsynch group). Group-I was subjected to estradoublesynch protocol where each animal was administered with $PGF_{2α}$ analogue (Cloprostenol @ 500 mcg; Inj. Estrumate 2 ml) intramuscularly (i.m.) on the start of treatment (Fig. 1). GnRH (gonadotropin releasing hormone) analogue (Buserelin acetate @ 20 mcg, Inj. Receptal, 5 ml) was given intramuscularly on day 2. On day 9, $PGF_{2α}$ analogue was again administered with same dose and route. On day 10, Estradiol Benzoate (@ 1 mg, Inj. Pregheat 1 ml, i.m.) was administered which was followed by fixed time artificial insemination (FTAI) at 48 h and 60 h (Mirmahmoudi *et al.,* 2014a). In Group II, the animals (n=10) were administered with GnRH analogue (Buserelin acetate @ 20 mcg, Inj. Receptal 5 ml i.m.) on day 0 and slow release intra vaginal progesterone device (CIDR, 1.38 g). On day 7, CIDR was

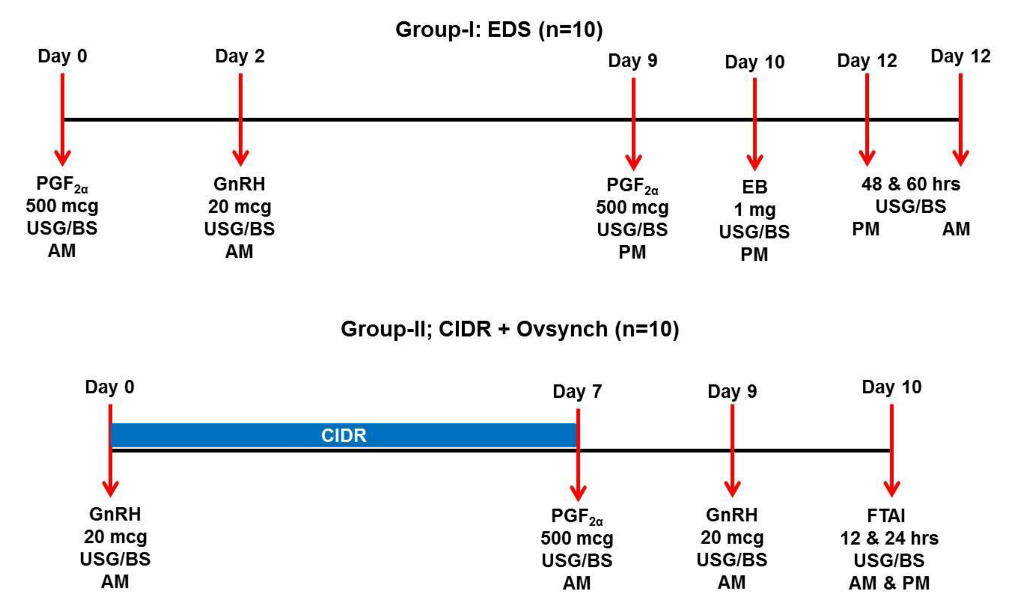

Fig. 1: Timeline for hormonal protocol in Group I and II (PGF2α: Prostaglandin, GnRH: Gonadotropin Releasing Hormone, EB: Estradiol Benzoate, FTAI: Fixed Time Artificial Insemination, USG: Ultrasonography, BS: Blood sampling, AM: Before midday, PM: After midday).

removed and $PGF_{2\alpha}$ analogue (Cloprostenol @ 500 mcg; Inj. Estrumate 2 ml) was administered. GnRH (Receptal @ 20 mcg, IM) was administered on day 9, followed by FTAI at 12 h and 24 h (Fig 1). Blood samples were collected by jugular venipuncture in heparinized vacutainer on different days of the treatment in both the groups (day 0, 9, 10, 12= Group-I and 0, 7, 9, 10= Group-II). Blood plasma was harvested and stored at -20°C till progesterone estimation. Similarly, the buffaloes were subjected to transrectal ultrasonographic examination on the aforesaid days in both the groups to record ovarian changes (number of follicles in different size categories and size of dominant follicle).

The plasma progesterone concentration was estimated using commercial progesterone estimation kit (Progesterone EIA, XEMA Co. Ltd., Russia) by plotting optical density (y-axis) and concentration of the calibrators (x-axis) on the standard curve drawn. The concentration of the test samples obtained in nanomoles, was converted into nanograms by using the conversion factor (1 nmol = 0.318 ng/ml).

The numerical data was represented as Mean ± SEM, and the differences were considered significant if $p<0.05$. Statistical analysis was performed using the standard procedure of SPSS software version 16.0. The data of various responses like estrus and ovulation induction were subjected to chi-square test. The Interval between application of $PGF_{2\alpha}$ to estrus appearance, the number of follicles of different size categories and plasma progesterone values at different occasions of treatment were analyzed using ANOVA and students' T test.

RESULTS AND DISCUSSION

The use of hormonal protocols *viz.* progesterone (CIDR) + Ovsynch protocol and estradoublesynch protocol in delayed pubertal buffalo heifers revealed outstanding estrus induction response. The estrus induction response was based on estrus-specific signs like bellowing, cervico-vaginal discharge, uterine tone and presence of dominant follicle as observed by trans-rectal ultrasonography. All the animals from both groups exhibited estrus and yielded 100% estrus induction rate. The present results agreed with the study reported by Kajaysri *et al.* (2015) where 100% estrus induction was achieved after the application of CIDR+ $PGF_{2\alpha}$+ GnRH protocol in anestrus postpartum buffaloes. Zarkar (2011) also observed 100 % estrus induction response in true anoestrus buffalo heifers after treatment with CIDR+ $PGF_{2\alpha}$+ GnRH. Dora (2016) observed 100% estrus induction response following estradoublesynch protocol in delayed pubertal cattle heifers. Furthermore, estrus induction response following the use of estradoublesynch and doublesynch protocols in anestrus Gir cattle during non-breeding season was reported as 95.0 and 85.0%, respectively (Chaudhary *et al.,* 2018).

The mean progesterone concentrations on the starting day of treatment were 0.49±0.1 and 0.4±0.04 ng/ml in group I and II animals, respectively. A significant rise ($P<0.05$) in P_4 concentrations was observed on day 9 in group I; however, the level declined on day 10 and day 12 (AI day) due to $PGF_{2\alpha}$. In group II animals, progesterone level significantly increased from day 0 of treatment (0.4±0.04 to 3.63±0.27) under the effect of exogenous P_4

Table 1: Mean±SEM values of plasma progesterone concentration (ng/ml) in Group I & II

Treatment groups	Treatment days				
	Day 0	Day 7	Day 9	Day 10	Day 12
Group I (EDS)	0.49^{AB}±0.10	-	0.83^{A} ±0.14	0.67^{A}±0.12	0.25^{B}±0.03
Group II (CIDR+ Ovsynch)	0.4^{A} ±0.04	3.62^{C} ±0.27	0.94^{B} ±0.18	0.29^{A} ±0.04	_

Level of significance P<0.05; where capital superscript indicates difference within group on different days

Table 2: Fertility response (%) following induced and spontaneous estrus in delayed pubertal buffalo heifers subjected to hormonal protocols.

Protocol	No. of heifers	Pregnancy rate at Induced estrus	Pregnancy rate at Spontaneous estrus		Overall Pregnancy rate
			First	Second	
(Group I) Estradoublesynch	10	50% (5/10)	0% (0/3)	33.33% (1/3)	60% (6/10)
(Group II) CIDR+ Ovsynch	10	20% (2/10)	28.57% (2/7)	60% (3/5)	70% (7/10)

Figures in parenthesis indicate number of buffaloes source i.e. CIDR. These levels gradually decreased to sub-basal levels (0.29±0.04) on day of AI (Table 1).

The use of estradoublesynch protocol in delayed pubertal buffalo heifers led to better ($p<0.05$) conception rates at induced estrus (50%) compared to CIDR+ Ovsynch protocol (20%; Table 2). But the overall conception rate was almost similar using the CIDR+ Ovsynch protocol to estradoublesynch protocol (70% *vs* 60%; Table 2). In previous studies, EDS yielded 54.5 % (Patel *et al.,* 2018) and 70% (Prajapati *et al.,* 2018) in anestrus buffaloes. Whereas CIDR+Ovsynch yielded 75% pregnancy rate in delayed pubertal cattle heifers (Bhutia, 2014) and 66.7% in sub estrus. Murrah buffaloes in low breeding period (Ghuman *et al.,* 2014). Zarkar (2011) reported an 83.33% conception rate following treatment with CIDR and GnRH in peripubertal buffalo heifers. Ghallab and Noseir (2016) reported an 80% conception rate following treatment with CIDR+Ovsynch in postpartum buffaloes. 35.7% of first service conception rate and overall conception rate of 50% followed by treatment with CIDR+Ovsynch in prepubertal buffalo heifers was reported by Ghuman *et al.* (2012). Conception rates of 60% (Mirmahmoudi *et al.,* 2014a) and 64% (Mirmahmoudi *et al.,* 2014b) following estradoublesynch protocol were reported in anestrus buffaloes.

CONCLUSIONS

In conclusion, estradoublesynch and progesterone-based ovsynch protocol can be effectively employed to induce ovulatory estrus and to improve pregnancy rates in delayed pubertal buffalo heifers.

CONFLICT OF INTEREST

The authors declare no conflict of interest among themselves.

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