Research Article

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BLOOD PLASMA PROGESTERONE AND ITS ABERRATIONS IN PUBERTAL BANNI BUFFALO HEIFERS FOLLOWING CIDR THERAPY

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ABSTRACT

The plasma progesterone (P_4) milieu during induced oestrus was studied in 22 pubertal Banni buffalo heifers treated for induced oestrus with 1.38 gm P_4 in CIDR implants for 9 days + 500 IU Folligon parentraly on day-1 (Group-I, n=8), CIDR + 500 IU PMSG (day -1) + 2.5 ml Receptal additionally at the time of observed oestrus (Group-II, n=8) and plain CIDR without any other treatment (Group-III, n=6). The blood samples from heifers of all three groups were collected on days -9, -5, -1, 0 (day of estrus) and thereafter on 5, 10, 15 20 and 30 days and plasma was obtained to quantify $P_4$by ELISA. The initialP_4 levels of <1 ng/ml on day of CIDR insertion (day -9) in all three groups varied non-significantly but the levels increased significantly (p<0.05) upto average of 11.35 ± 2.12 and 12.05 ± 0.90 ng/ml on day of CIDR withdrawal (day -1) in Group I and II, respectively while in Group III, it continued to be<1 ng/ml. The lowest P_4 levels in the heifers of both treatment groups were recorded on day of induced estrus which again increased upto day 30 with maximum concentrations of 10.65 ± 0.78 and 11.77 ± 0.79 ng/ml in Group I and II, respectively to differ significantly (p<0.05) than that of Group III (0.33 ± 0.10 ng/ml). 3 out of 16 heifers (18.75 %) had the P_4 aberrations in the form of levels < 1 ng/ml during the periods of CIDR insertion and withdrawal in two heifers, one each of Group I and II, whereas remaining heifer in Group II had elevated P_4 level of 1.4 ng/ml at induced estrus which later dropped to <1ng/ml during post-estrus period up to day 30. The P_4 aberrations were coupled with conception failure. It is concluded that circulatory P_4 remained normal in majority of heifers with few exceptions of low or high P_4 levels at certain intervals of the CIDR treatment.

Key words : Bunni buffalo, CIDR, Progesterone

Bunni buffalo is known for higher productivity, disease resistance and better adaptation in arid climate. It also possesses a unique potential of reproductivity as the service period and calving interval are of shorter duration than other breeds of buffalo (Singh, 1992; Chavan, 2006). However, the problem of delayed puberty is encountered generally which curtails the calf crop and life time milk production. The hormone alone or incombination (Anderkar and Kadu, 1995;Patel *et al*., 2003; Azawi *et al*., 2012) and the herbal medicines (Panchal *et al*., 1996) have been reported as remedial measures of such animals, but the fertility response varied with *in-situ* duration of hormone and its combinations (Saini *et al*., 1988; Markandaya *et al*., 2002; Bartolomeu *et al.,* 2007). Therefore, the efficacy of different hormone protocols to induce ovarian activity was assessed in the pubertal Banni heifers by monitoring the plasma P_4 profiles.

MATERIALS AND METHODS

The present study was carried out on twenty two Bunni buffalo heifers of pubertal age but failed to express behavioral signs of oestrus apart from ovarian cyclic changes upto age of > 30 to 36 months. The heifers were selected randomly at the Cattle Breeding Farm, Kutchch and maintained under uniform managemental practices. All heifers were divided into three different groups, one group to receive 1.38 gm progesterone in controlled internal drug release device (CIDR, EAZI-BREED®, Pfizer, Animal Health, New Zealand) for a period of 9 days followed by parentral administration of 500 IU pregnant mare serum gonadotropin (PMSG, Folligon®, MSD Animal Health, Pune) on day of CIDR withdrawal (Group-I, n=8). The heifers allotted to Group-II (n=8) also received the similar treatment but were injected additionally a single shot of gonadotropin releasing hormone (GnRH, Receptal®, 0.0042 mg/ml, MSD Animal Health, Pune) at the time of observed induced oestrus whereas only plain CIDR were advocated to the control heifers (Group-III, n=6). The blood samples from heifers of all three groups were collected in K_3EDTA vials through jugular vein on day -9, -5, -1, 0 and thereafter on 5, 10, 15, 20, and 30 days following withdrawal of CIDR implant considering the day 0 as day of induced oestrus. The plasma obtained by centrifugation of all samples at 2000 rpm was stored at -20°C. The progesterone hormone (P_4) was quantified by enzyme linked immune-sorbent assay (ELISA) using commercially available kits (Novatec Immunodiagnostic GmbH, Germany). The standard curve was prepared using P_4 standards on ELISA reader (Multi Scanner, Thermo Scientific) and hormone concentration was calculated accordingly. The sensitivity of kit was 0.05 ng/ml at the 95% confidence limit. The data were analyzed through one way ANOVA and the treatment mean values were compared using Duncan's Multipale Range Teat (Snedecor and Cochran, 1985).

RESULTS AND DISCUSSION

The mean values of plasma P_4 in treatment and control groups are presented in Table 1. The initial progesterone levels <1ng/ml just prior to insertion of CIDR i.e. on day -9 were observed in both treatment and control group heifers. The differences of mean values were non-significant and reflected the non-functional state of

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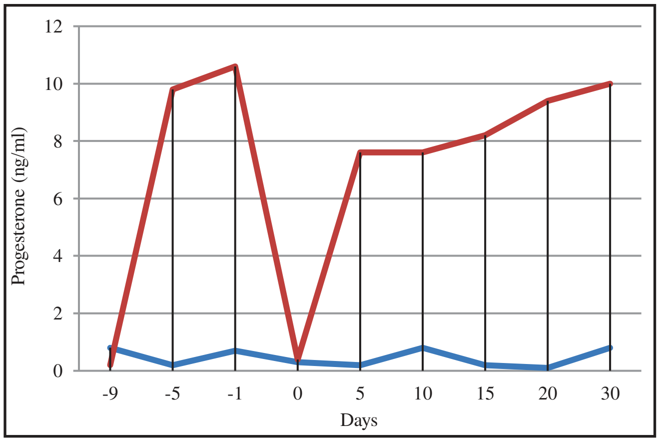

Fig. 1. Aberrant progesterone profile in non-conceived buffalo heifers (Group-I)

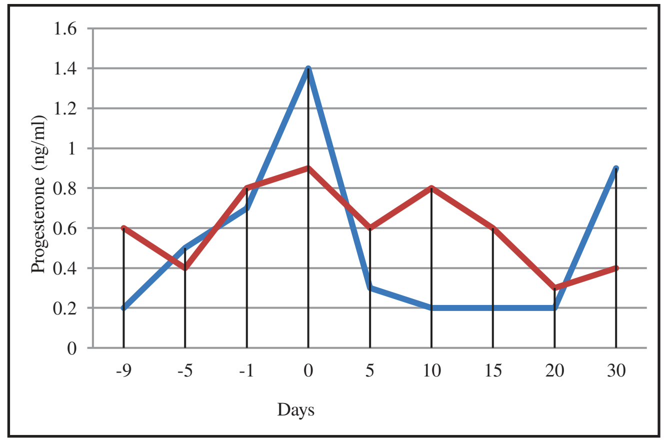

Fig. 2. Aberrant progesterone profile in non-conceived buffalo heifers (Group-II)

ovaries in all three groups. These findings are in accordance with the observations of Sharma *et al.* (1999), Kumar *et al.* (2005), Caeser *et al.* (2011) and Ghuman *et al.* (2012). Following insertion of CIDR, the P_4 concentrations significantly increased than that of initial value and continued to be higher consistently during the period of implant insertion and attended the levels of 11.35±2.12 and 12.05±0.90ng/ml on day-1 in both treatment groups, whereas no such increase of plasma P_4 concentration was discernable in heifers of control group and levels were observed to be <1ng/ml (Table 2).The increased concentrations differed significantly than their preceding levels in both treatment groups. Subsequently the hormone level declined to its lowest average of 0.61± 0.28 and 0.35±0.23ng/ml on day of estrus and again started to increase upto day 30 to its maximum levels of 10.65 ± 0.78 and 11.71±0.79ng/ ml in Group-I and II, respectively. Elevated P_4 concentration with a maximum level of 19.15±3.30ng/ml in heifers following insertion of CIDR implants was also reported earlier by Singh *et al.* (2006) whereas other workers reported circulatory progesterone concentration of >1ng/ml (Burke *et al.*, 1999; Nation *et al.*, 2000; Mann *et al.*, 2001) which might be associated with type and duration of P_4 therapy (Andurkar *et al.*, 1997) increased dose of P_4 in drug releasing device (McDongall *et al.*, 2004). However, the consistently increased level of P_4 during the period of CIDR insertion observed in the present study confirmed its positive effect on the follicular growth and diameter of the dominant follicle as reported earlier by SaFilho *et al.* (2010).

Table 1
Progesterone concentration (Mean±SE) in Banni buffalo heifers of treatment and control groups

Interval (Days)	Progesterone (ng/ml)		
	Group -I	Group -II	Group -III
↓ -9	0.30±0.31[a]	0.60±0.26[a]	0.36±0.17[a]
-5	9.63±1.24[cde]	10.83±0.90[fgh]	0.40±0.16[a]
↑ -1	11.35±2.12[ghi]	12.05±0.90[i]	0.38±0.11[a]
0	0.61±0.28[a]	0.35±0.23[a]	0.43±0.16[a]
5	8.85±1.52[c]	7.71±1.36[b]	0.40±0.18[a]
10	9.25±1.34[cd]	10.03±1.64[def]	0.28±0.14[a]
15	9.60±0.82[cde]	10.61±0.67[efg]	0.48±0.13[a]
20	10.25±0.76[defg]	11.01±0.21[fghi]	0.40±0.17[a]
30	10.65±0.77[efg]	11.76±0.78[hi]	0.33±0.10[a]

In the treatment groups, the P_4 concentrations <1 ng/ml were recorded following withdrawal of CIDR implant; and it was the period where majority of the heifers showed induced oestrus, hence, the low progesterone is quite expected at this time. These findings are in accordance with earlier reports (Chauhan *et al.*, 1985; Sharma *et al.*, 1999; Singh and Madan, 2000; Chaudhari, 2006). Low progesterone at 72 hour post-withdrawal of CIDR coincided with period around the induced oestrus was also reported by Singh *et al.* (2006).

Whereas P_4 levels remained low at induced oestrus, the post-oestrus findings revealed its elevated concentrations of 10.65±0.77 and 11.76±0.78ng/ml in treatment Group-I and II, respectively by day 30 of induced oestrus. On the other hand, no such increase in P_4 concentration was inferred from the findings in the control group. Further, the comparison of progesterone levels between the treatment and control Groups revealed approximately 10 time higher levels on day 30 in treated heifers (10.65±0.77, Group-I; 11.76±0.78ng/ml, Group-II vs 0.33±0.10ng/ml, control Group-III). Such a post-oestrus rise of progesterone levels was indicative of ovulation in treatment groups. Anoestrus buffalo heifer treated with CIDR+PMSG regimen were also reported to have elevated progesterone level (4.20±1.60ng/ml) on day 10 of induced oestrus(Caeser *et al.*, 2011). Higher P_4 during luteal phase (Bansal *et al.*, 2004), pregnancy (Abou-Elo-Roos and Abdel Gaffar, 2000; Kumar *et al.*, 2010) and day 16 of post-oestrus with Crestar and PMSG (Sarmah *et al.*, 2008).

Apart from increased P_4 profile during the period of CIDR implants, lowest hormone at induced oestrus and

Table 2.
ANOVA of Progesterone in Banni buffalo heifers of treatment and control groups

Source of variance	df	SS	MS	Cal F
Treatment	2	473.907	236.953	321.41*
Interval	24	1892.344	78.847	106.95*
Error	171	99.525	0.7372	
Total	197	4130.768	25.6569	

again rise during post-oestrus periods in both treatment groups, few aberrations of progesterone hormone *vis-a-vis* conception failure were also discerned in 3 out of 16 heifers (18.75 %) of present study. Of these, one heifer each in Group-I and II was observed to have low P_4 (<1 ng/ml) during the entire course of study. It might be explained by elevated levels of serum specific proteins during the treatment which bind to P_4 and keep the unbound and active hormone low in circulation (Mestman and Nelson, 1963). The findings of Sikka *et al*. (1993) also confirm the possible role of binding proteins in maintaining the correct proportion of circulatory level of progesterone at a given time. On the other hand, the failure of another heifer to conceive even after progesterone milieu similar to that of conceived heifer was observed in Group-I (Fig. 1), which might be due to reduced fertility on account of extended or prolonged life span of dominant/persistent follicle (Beal *et al*., 1998) and ovulation of subfertile oocyte as reported earlier in cows (Ahmed *et al*., 1995). Further, one more heifer in Group-II did not reveal rise of progesterone during the CIDR insertion and post-induced oestrus but had the elevated progesterone hormone >1 ng/ml on day of induced oestrus (Fig. 2) which might be attributed to premature ovulation of dominant follicle during the course of P_4/CIDR priming and its withdrawal as reported earlier by Mann and Lamning (2000).

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