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Oestrus Induction, Plasma Steroid Hormone Profiles and Fertility Response after CIDR and eCG Treatment in Acyclic Sahiwal Cows

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ABSTRACT : The study was conducted on 30 true acyclic Sahiwal cows (15 cows, ≥90 days postpartum; 15 postpubertal heifers, ≥30 months of age) and a similar 20 untreated controls (10 cows, 10 heifers). An ‘Eazi’ breed Controlled Internal Drug Release (CIDR) device (containing 1.38 g progesterone) was inserted intravaginally for 7 days (days 0 to 7) followed by 500 IU eCG i.m. at CIDR removal in all the treated animals. Heifers also received 5 mg oestradiol valerate i.m at CIDR insertion. The reproductive performance of these animals was recorded in terms of oestrus induction response, conception and pregnancy rates. Plasma progesterone (P_4) and oestradiol-17β (E_2) profiles of 4 representative animals from each treatment group before, during and after CIDR treatment were also monitored. An oestrus induction response of 100% was observed in treated cows and heifers. The majority of cows (53.3%) and heifers (60%) were induced to oestrus within 24-36 and 36-48 h, respectively after CIDR withdrawal; with mean intervals of 44±3.18 and 48±2.35 h, respectively. The conception rate at induced oestrus was higher in cows (40%) than heifers (20%). The final pregnancy rates after 2 subsequent oestruses were 80 and 60% in cows and heifers, respectively (overall 70% for all treated animals). In comparison, only 10% of control animals (2 cows only, 2/20) showed oestrus and become pregnant (10%) during theentire study period. The pretreatment (day 0) mean plasma P_4 levels were statistically ($p>0.05$) similar in cows and heifers (0.40±0.04 and 0.49±0.11 ng/ml, respectively). The peak P_4 levels were observed on day 1 in cows (13.94±1.41 ng/ml) and day 2 in heifers (19.15±3.30 ng/ml) with a progressive decline up to the day of CIDR withdrawal (3.35±0.92 and 8.79±1.71 ng/ml, respectively). Mean P_4 levels on day 9 and 10 in cows and heifers did not differ significantly from their respective day 0 values and the lowest values were recorded on day 10 both in cows and heifers (0.13±0.03 and 0.14±0.02 ng/ml, respectively). Wide variations in individual pretreatment E_2 levels were observed both in the cows (range = 4-26, mean = 13.00±4.65 pg/ml) and heifers (range = 10-14, mean = 11.50±0.96 pg/ml). Thereafter also, E_2 levels in cows showed variation and reached a peak level (53.50±2.99 pg/ml) on day 8. In heifers, peak mean E_2 level (111.25±39.81 pg/ml) was recorded on day 1, followed by a non-significant decline on day 2, a significant fall on day 6 and a non-significant increase on day 9 and 10. However, mean E_2 levels on days 7 ($p<0.05$), 8 and 9 ($p<0.01$) were significantly higher in cows compared to heifers. The post-CIDR withdrawal mean highest P_4 and lowest E_2 levels coincided with the period when the majority of animals were induced to oestrus. CIDR and eCG treatment resulted in effective induction of oestrus with satisfactory pregnancy rates in true acyclic Sahiwal cows and heifers. (**Key Words :** CIDR, Acyclic, Oestrus Induction, Steroid Hormone, Fertility, Sahiwal Cows)

INTRODUCTION

Anoestrus resulting from delayed postpartum conception in cows and delayed onset of cyclicity in heifers is the major contributor to infertility in Indian cattle, which accounts for poor reproductive efficiency of Zebu cattle. It leads to increased calving interval (Roche et al., 1992) and hinders milk and meat production. The minor factors such as season, breed, parity, dystocia, presence of bull, uterine palpation and carryover effects from the previous pregnancy and the major factors, i.e. suckling and lactation affects the postpartum fertility (Short et al., 1990). Apart from these, short oestrous cycles also contribute to infertility in about 50-80% of postpartum cows (Webb et al., 1980; Bulman and Laming, 1989), particularly during the first 30-40 days postpartum (Short et al., 1990). The short luteal phase following the first spontaneous postpartum ovulation is a consequence of interactions between the uterus, corpus luteum, and possibly the ovulatory follicle (Cooper et al., 1991). These short cycles provide

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progesterone (P_4) exposure for a short period and elevated P_4 levels during the postpartum period, either from endogenous or exogenous sources is important for the expression of oestrus as well as subsequent normal luteal function (McDougall et al., 1992).

Maintaining subluteal or intermediate circulating concentrations of plasma P_4 by exogenous P_4 administration mimics such short oestrous cycles and helps to maintain the dominant follicle of the cycle (Yavas and Walton, 2000), via enhancing the release of LH and stimulating development of LH receptors and oestradiol secretion (Day and Anderson, 1998).

Exogenous P_4 administration induces cyclicity in a substantial proportion of cows within few days of its withdrawal (Day, 2002). Intravaginal P_4 releasing devices have been used mainly for the synchronization of ovulation and oestrus but are less applied for induction of cyclicity in true acyclic cows and heifers. Various research workers (Hansel and Beal, 1979; Belloso et al., 2002) have obtained satisfactory induction of oestrus and ovulation in postpartum Zebu cows using intravaginal P_4 administration and calving rates can further be improved by addition of eCG at the end of P_4 therapy (Munro and Moore, 1985).

In view of this, Controlled Internal Drug Release (CIDR) device may hold the potential of enhancing the reproductive performance by combating the problem of anoestrus in postpartum cows and postpubertal heifers. In India, fragmented research work has been done to evaluate the efficacy of CIDR for induction of cyclicity in Indigenous cattle. Moreover, changes in steroid hormone profiles during induction of cyclicity with natural P_4 have also not been well documented.

MATERIALS AND METHODS

Healthy Sahiwal cows (n = 15) with normal genitalia (≥90 days postpartum, true acyclic since parturition, group I) and healthy postpubertal Sahiwal heifers (n = 15) with normal genitalia (≥30 months of age, acyclic since attainment of sexual maturity, group II) were selected as treatment animals after confirming as true acyclic by 2 per-rectal examination of ovaries at 10 days interval. Control group Sahiwal cows (n = 10) and postpubertal Sahiwal heifers (n = 10) were also selected similarly and kept as untreated controls. Both treatment and control animals were dewormed 15 days prior to experiment and allotted identification numbers and were kept in the herd itself.

All the cows and heifers selected for the study were kept under isomanagerial conditions. All the treatment animals (group I and II) received 'Eazi' breed CIDR™ (containing 1.38 g of natural P_4) intravaginal device (InterAg, Hamilton, New Zealand) on day 0 for seven days (days 0 to 7). In addition, group II (heifers) also received 5 mg of oestradiol valerate (Progynon Depot™, German Remedies, Mumbai, India) i.m. at the time of CIDR insertion. Animals of both the groups received 500 IU of PMSG (Folligon™, Intervet International BV, Boxmeer, Holland) i.m. at the time of CIDR device withdrawal. After CIDR withdrawal on day 7, all the animals were observed for oestrus starting from 6 h post-CIDR withdrawal. Animals mounting or standing to be mounted or being teased or mounted by teaser bull were confirmed to be in oestrus by rectal palpation. Based on this data, distribution of oestrus response (interval from CIDR removal to onset of induced oestrus) was calculated. Animals confirmed in oestrus were artificially inseminated twice, at 12 h after onset of oestrus (1st AI) and again at 12 h later (2nd AI) using thawed-frozen semen.

Blood samples were collected by jugular venu-puncture from randomly selected 4 treatment cows and 4 heifers before CIDR insertion (day 0), during period of CIDR insertion (days 1 to 6) and during the period of induced oestrus and AI (days 7 to 10). Blood plasma was harvested by centrifugation at 3,000 rpm for 10 min, aliquoted in duplicate and stored at -20°C till hormone assay. Plasma P_4 was analyzed by radio-immuno assay (RIA) kit (Immunotech, Marseille, France) on days 0 to 10 as per protocol recommended by manufacturer in a single assay. The lower limit of sensitivity of the assay was 0.03 ng/ml and the intra-assay coefficient of variation ranged between 4.32 and 6.87%. Plasma oestradiol-17β (E_2) was also estimated by using RIA kit (Immunotech) on days 0, 1, 2, 4, 6, 7, 8, 9 and 10 as per protocol recommended by manufacturer. The sensitivity of assay was 4.0 pg/ml and the intra-assay coefficient of variation ranged between 8.82 to 13.19%. The inter-assay coefficient of variation was 6.67 to 16.18%.

Statistical analysis of fertility response data, P_4 and E_2 levels was carried out by using analysis of variance (ANOVA), Duncan's multiple range test and student '*t*' test as described by Snedecor and Cochran (1994).

RESULTS

Induction of oestrus and pregnancy rates

All the CIDR treated cows (15/15) and heifers (15/15) were induced to oestrus within 24 to 60 h post-CIDR withdrawal period whereas, in the control group only 2 cows (2/10) and no heifer (0/10) showed oestrus during the study period.

Fifty three percent treated cows (8/15) and 20% of the treated heifers (3/15) exhibited onset of induced oestrus between 24 to 36 h post-CIDR withdrawal, whereas 60% heifers (9/15) and only 27% of cows (4/15) showed oestrus between 36 to 48 h post-CIDR withdrawal. Twenty percent

Table 1. Distribution of oestrus response in CIDR treated cows (group I) and heifers (group II)

Treated animals	Total no. of animals	No. in induced oestrus (h post CIDR removal)			Mean(±SE) interval to onset of induced oestrus (h)
		24-36	36-48	48-60	
Cows (group I)	15	8 (53.33)	4 (26.66)	3 (20)	44.00±3.18
Heifers (group II)	15	3 (20)	9 (60)	3 (20)	48.00±2.35
All cows and heifers (group I+II)	30	11(36.66)	13 (43.33)	6 (20)	46.00±1.97

* Figures in parenthesis indicate percentage.

Table 2. Fertility status of control and CIDR treated cows and heifers

Number of animals	Treated		Control	
	Cows (n = 15)	Heifers (n = 15)	Cows (n = 10)	Heifers (n = 10)
Induced to oestrus after CIDR withdrawal	15 (100%)	15 (100%)	2 (20%)	-
Inseminated at induced oestrus	15	15	2	-
Pregnant at induced oestrus	6 (40%)	3 (20%)	2 (20%)	-
Pregnant at subsequent oestruses	6	6	-	-
Total pregnant	12 (80%)	9 (60%)	2 (20%)	-

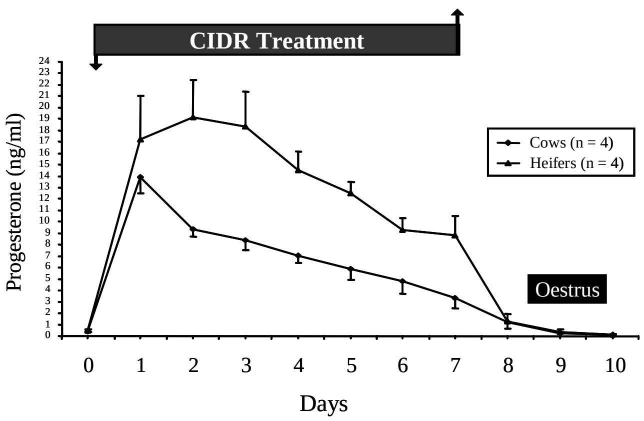

Figure 1. Mean (±SE) plasma progesterone concentrations in CIDR treated cows (group I) and heifers (group II).

each of cows (3/15) and heifers (3/15) showed oestrus in 48 to 60 h post-CIDR withdrawal periods. Cows had a slightly lower (non-significant, p>0.05) mean interval to onset of induced oestrus (44±3.18 h) compared to heifers (48±2.35 h), with an average mean interval of 46±1.97 h for all treated animals (Table 1).

Since both in group I and II, the oestrus induction was 100%, AI was done twice in all the treatment animals at the induced oestrus. Those failed to conceive and showed oestrus upto 2 subsequent cycles were again double inseminated. Thus fertility status was studied for one induced and 2 subsequent oestrous cycles. A total of 40% cows (6/15) and 20% heifers (3/15) conceived at the induced cycle and other 40% each of cows (6/15) and heifers (6/15) settled during the 2 subsequent cycles. Thus, an overall pregnancy rate of 80% in treated cows and 60% in heifers was achieved. In comparison, only 10% control animals (2 cows only) were observed in oestrus with a similar pregnancy rate (10%, 2/20) during this period of study (Table 2).

Steroid hormone profiles

The pretreatment (day 0) plasma P_4 levels both in group I (cows) and II (heifers) were below 0.5 ng/ml except in one heifer (0.8 ng/ml). The mean day 0 concentrations in both the treatment groups were statistically similar (0.40±0.04 and 0.49±0.11 ng/ml, respectively, p>0.05). At 24 h post-CIDR insertion, mean P_4 levels registered a significant (p<0.05) increase in cows (13.94±1.41 ng/ml) and heifers (17.20±3.84 ng/ml). Highest mean P_4 levels were recorded on day 1 in cows (13.94±1.41 ng/ml) and day 2 in heifers (19.15±3.30 ng/ml). Thereafter, a progressive decline in P_4 levels was recorded till CIDR withdrawal in both the groups (Figure 1). However, the decline between mean peak level and succeeding day value was sharp and significant in cows compared to heifers. Further decline was steady and gradual in both the groups (Table 3). Despite of continuous plasma P_4 decline, its mean levels on CIDR withdrawal (day 7) were significantly (p<0.01) higher both in the treatment cows (3.35±0.92 vs. 0.40±0.04 ng/ml) and heifers (8.79± 1.71 vs. 0.49±0.11 ng/ml) compared to their respective day 0 levels. The mean P_4 levels in the cows and heifers were similar at 24 h post-CIDR withdrawal (1.19±0.53 and 1.27±0.68 ng/ml, respectively). Post device withdrawal (day 8) individual P_4 levels varied widely both in the cows and heifers and most of the animals had uniformly low levels on day 9 and 10. At 48 h post-CIDR removal, levels had reached to 0.24 ng/ml in cows and 0.37 ng/ml in heifers and the lowest values of 0.13 and 0.14 ng/ml, respectively were recorded at 72 h post-CIDR withdrawal. These lowest P_4 levels coincided with the period around the time of induced oestrus.

The pretreatment (day 0) mean basal plasma E_2 levels showed a wide variation from 4 to 26 pg/ml in cows and a less variation from 10 to 14 pg/ml was observed in heifers. On day 1, the mean E_2 level in heifers significantly

Table 3. Mean (±SE) plasma progesterone and oestradiol-17β concentrations in CIDR treated cows (group I) and heifers (group II)

Animal		Days										
		0	1	2	3	4	5	6	7	8	9	10
Progesterone (ng/ml)	Cows (group I)	0.40^{h} ±0.04	13.94^{a} ±1.41	9.36*^{b} ±0.68	8.38*^{bc} ±0.84	7.07**^{cd} ±0.72	5.88**^{de} ±0.98	4.78*^{ef} ±1.09	3.35*^{fg} ± 0.92	1.19^{gh} ±0.53	0.24^{h} ±0.02	0.13^{h} ±0.03
	Heifers (group II)	0.49^{e} ±0.11	17.20^{ab} ±3.84	19.15*^{a} ±3.30	18.33*^{a} ±3.03	14.50**^{abc} ±1.66	12.52**^{bcd} ±0.97	9.28*^{cd} ±1.02	8.79*^{d} ±1.71	1.27^{e} ±0.68	0.37^{e} ±0.22	0.14^{e} ±0.02
Oestradiol (pg/ml)	Cows (group I)	13.00^{d} ±4.65	12.50*^{d} ±3.40	21.00*^{cd} ±0.70	-	45.00^{ab} ± 10.26	-	34.25*^{bc} ±4.03	31.25*^{bc} ±4.64	53.50**^{a} ±2.99	53.00**^{a} ±6.72	32.00^{bc} ±10.17
	Heifers (group II)	11.50^{b} ±0.96	111.25*^{a} ±39.81	74.75*^{a} ±17.44	-	27.50^{a} ±9.03	-	18.50*^{b} ±3.10	17.00*^{b} ±1.73	17.25**^{b} ±1.70	25.75**^{b} ±1.55	24.50^{b} ±1.50

*, ** Show difference between means of cows and heifers for progesterone as well as oestradiol on a day (*$p<0.05$, ** $p<0.01$, respectively).
Means within a row with different alphabet superscripts differ significantly ($p<0.05$).

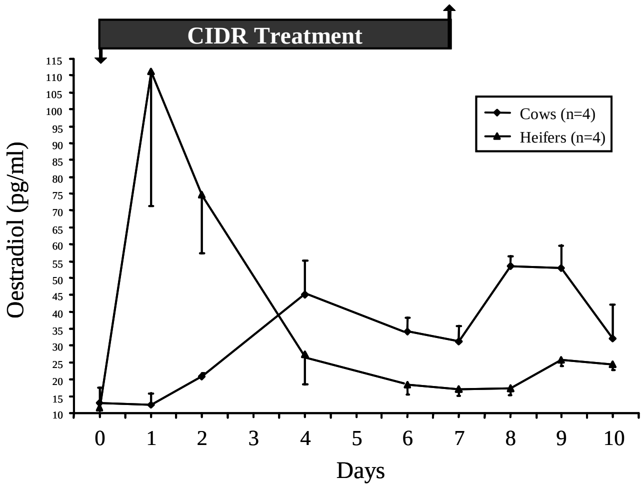

Figure 2. Mean (±SE) plasma oestradiol-17β concentrations in CIDR treated cows (group I) and heifers (group II).

increased from their mean basal value of 11.5±0.96 to 111.25±39.81 pg/ml whereas in cows there was a non-significant variation (13±4.65 and 12.50±3.40 pg/ml, respectively, $p>0.05$) only. In heifers, there was a decline in E_2 concentrations from day 4 to 8 (Figure 2). In cows, a slight elevation in mean E_2 level was observed on day 2 over the day 1 (21±0.70 vs. 12.50±3.40 pg/ml), followed by a sharp and significant ($p<0.05$) increase on day 4 (45±10.26 pg/ml). Thereafter, a non-significant decline ($p>0.05$) was observed on day 6 and 7 (Table 3). Further, mean E_2 levels in cows showed a significant increase on day 8 (53.50±2.99 pg/ml), however, on day 10, the E_2 levels in cows again registered a significant decline (32.00±10.17 vs. 53.00±6.72 pg/ml) in comparison to day 9 values. On day 10, mean E_2 levels in cows and heifers were similar (32±10.17 and 24.15±1.50 pg/ml, respectively). The mean E_2 levels in cows on days 7 ($p<0.05$), 8 and 9 ($p<0.01$) were significantly higher than in heifers. The mean interval to onset of induced oestrus in cows was non-significantly higher (44±3.18 vs. 48±2.35 h) than in heifers.

DISCUSSION

In India, various indigenous breeds of cattle have poor reproductive performance as compared to crossbreds and European breeds. Late maturity and long inter-calving intervals are the major factors resulting in poor fertility of Zebu cattle. Irregularity in cyclicity during long postpartum periods in cows and acyclicity in heifers even after attaining of pubertal age and body weight need to be managed for good economical gains to the dairy farmers on a sustainable basis.

The present study was an attempt for the reproductive management of acyclic cows and heifers by substituting exogenous administration of synthetic progestogens by natural P_4 (CIDR), while keeping the use of oestradiol valerate (OV) optional. Keeping in view the possible detrimental effects of E_2 on milk yield, the same was given to group II (heifers) only.

All the Sahiwal cows (group I) and heifers (group II) treated with CIDR and eCG in the present study were induced to oestrus. Earlier studies using Crestar or CIDR in combination with small doses of E_2 and eCG in Sahiwal (Singh et al., 1998) and Nelore cows (Barufi et al., 2002) have highlighted the possible beneficial effect of such therapies. Our study agreed with Saraswat et al. (1997), as they also reported 100% oestrus induction both in acyclic cows and heifers with a 9 day CIDR regimen alongwith 1,000 IU of eCG on device removal whereas, oestrus response of 93.3% in cyclic (Kacar and Aslan, 2004) and 75% in acyclic cows (Takagi et al., 2005) using CIDR have also been reported. E_2 administration at the time of CIDR insertion was reported (Ryan et al., 1999) to lower the oestrus induction in anoestrus cows. Contrary to this, no such effect was observed in this study as all heifers (group II) received OV and all were induced to oestrus. However, 75% of oestrus induction response (Gonzalez et al., 1998) and an improvement in pregnancy rates (McDougall, 2001) by giving oestradiol benzoate (OB) at CIDR insertion in anoestrus cows have also been reported.

The observation of a majority of animals exhibiting oestrus within 36 h post-device removal strongly contradicts the recommended fixed-time insemination schedule. Even breeding at the observed oestrus resulted in rather poor first-service conception rates in the present study, which could have been further impacted by timed inseminations. The stage of cycle at the beginning of treatment is an essential factor determining the interval to oestrus after device removal (Macmillan and Peterson, 1993). Since in the present study, all females were supposed to be known acyclic at the start of treatment, a reasonably better synchrony was achieved with a majority of animals in the oestrus within 48 h. Saraswat et al. (1997) and Kacar and Aslan (2004) had obtained a longer mean interval (>80 vs. 44 h) to induced oestrus in cows using CIDR compared to the present study. In the present study, heifers took nearly 4 h longer time to exhibit cyclicity compared to cows. However, Slightly longer interval to onset of oestrus in virgin heifers (Garcia and De Jarnette, 2003) and on contrary a shorter interval to oestrus in subfertile cows (Terui et al., 2003) have been reported on giving a dose of E_2 at CIDR insertion compared to animals not administered E_2. Hanlon et al. (1996) observed that administration of 0.5 mg of OB 24 h after CIDR removal in heifers significantly improved the onset of oestrus and the percentage of heifers exhibiting oestrus within 48 h of device removal with no effect on conception rates. Later in 1997, they reported that post-CIDR removal OB administration induced an early LH peak in all animals without any significant effect on the occurrence of standing oestrus. In the present study, only heifers were administered 5 mg OV at the time of CIDR insertion without any additional dose post-CIDR removal. Out of 15, twelve heifers exhibited oestrus within 48 h of CIDR withdrawal that perhaps is the optimum time for breeding of synchronized cows.

Interestingly, after a low conception at induced oestrus, cyclicity was maintained to 2 subsequent cycles both by the cows and heifers, giving satisfactory overall pregnancy rates. The conception rates in animals after induction/synchronization with natural/synthetic P_4 regimes are generally reported to be better in heifers than parous females (Saraswat et al., 1997; Singh et al., 1998). However, contrary to this, in the present study, both the induced oestrus and overall pregnancy rates were lower in heifers (group II) compared to cows (group I). A synchronized conception rate of 52% in CIDR treated heifers was reported by Hanlon et al. (1996). Comparatively, higher synchronized conception rates (Ryan et al., 1999) in cows and lower pregnancy rates in acyclic heifers (Henry et al., 2003) using CIDR alongwith $PGF_2\alpha$ and GnRH have also been reported. Xu et al. (2000) also reported a higher fertility response in non-cyclic cows with the same combination. Earlier Macmillan and Pickering (1988) had reported a 70% oestrus response and 60% conception rate at the induced oestrus in acyclic dairy cows. The overall pregnancy rates in cows (80%) and heifers (60%) after CIDR treatment in the present study compares favourably with these findings.

The pretreatment (day 0) plasma P_4 levels (<0.5 ng/ml) in treatment cows and heifers, suggests ovarian inactivity and hence acyclicity, except in one heifer (0.8 ng/ml) which could either be accounted for an erroneous clinical diagnosis or more likely to an extragonadal P_4 source (Madan, 1988). The low plasma P_4 profiles in acyclic bovine females have been widely reported (Savio et al., 1990; McDougall et al., 1995; Singh et al., 1998). Kerr et al. (1991) observed that in about 36% of clinical anoestrus heifers, P_4 levels did not corroborate their non-cyclic status. Irrespective of basal P_4 levels, sharp increase in these values on day 1 was observed in all the treated cows and heifers. P_4 concentrations exhibited a declining trend from day 2 in cows, whereas most of the heifers had peak values on day 2 and one animal on day 3. This indicates a comparatively slow vaginal absorption of P_4 in heifers and progressive decline in heifers started on day 3 or 4 onwards. Both in the cows and heifers, inspite of wide variation in individual P_4 values on day 8, mean levels on day 9 and 10 were similar to ones expected around the oestrus.

Burke et al. (1999) recorded high plasma P_4 level of 10 ng/ml at 2 h post-CIDR insertion in cows, which remained elevated over the next 2 or 3 days before a decline was recorded. Mann et al. (2001) also obtained high plasma P_4 levels (10.3±0.8 ng/ml) using half-strength CIDR devices. Therefore, peak values both in cows and heifers of our study on days 1, 2 or 3 are not dissimilar to earlier studies. Similarly, high P_4 concentration at 24 h post-CIDR insertion in cows were also reported by Nation et al. (2000) and Xu et al. (2000) which declined over the period of device insertion with values touching base levels subsequently. An almost similar pattern of plasma P_4 concentrations was recorded in the present investigation, except that levels in the present study were much higher. Elevated plasma P_4 levels to near luteal levels (5-7 ng/ml) by 24 h post-CIDR insertion in ovariectomized cows, declining 2-3 days later to 2-3 ng/ml and then remaining at the these levels until CIDR removal (day 7) agreeing with our hypothesis have also been reported (Martinez, 2002). It is apparent, therefore, that high plasma P_4 levels maintained by CIDR for about a week (7 day device insertion) were probably sufficient stimulus to trigger these non-cyclic females into cyclicity, probably by resultant feed back system of P_4 on the gonadotropic system. The necessity of low basal P_4 levels during the peri-oestrual days was within this range on day 9 and 10, which possibly would be a contributive factor for the excellent oestrus response obtained.

The almost similar mean basal E_2 values in the 2 treatment groups appear to be confirmative of their acyclic status. Similar mean E_2 concentration in non-cyclic Sahiwal heifers (13.7±1.62 pg/ml) and cows (14.56±0.78 pg/ml) has been reported (Singh, 1995). Higher pre-treatment E_2 concentrations in postpartum non-cyclic cows have been reported by Rao et al. (1981). Madan et al. (1983) reported plasma E_2 levels ranging between 10 to 37 pg/ml in true anoestrus cattle. Nath et al. (2003) also reported mean E_2 concentration of 18.33 pg/ml in anoestrus cows of Assam which shot up to 70 to 80 pg/ml after treatment with norgestomet implants. Therefore, from the scanty literature available, it can perhaps be affirmed to be representing the basal E_2 values. Yet, further investigations on a larger numbers of animals seem essential to confirm whether these values actually reflect the basal E_2 levels in Sahiwal breed. The immediate increase in E_2 levels on day 2 post-device insertion in heifers is a reflection of the exogenous E_2 administered in this group. Similar findings have been reported by others (Uehlinger et al., 1995; Martinez et al., 2005) using PRID or CIDR in combination with E_2 administration in cows. However, in cows increase in E_2 levels from day 2 onwards is assumed to be the result of a possible wave of emerging follicles. Post-device removal, the E_2 levels further increased to 53 pg/ml on day 8 and 9, a period in which 80% (12/15) animals exhibited oestrus. It is also postulated that since 40% treated females conceived at the induced oestrus, the reasonably high E_2 peaks would have induced appropriate LH peaks to bring about the ovulatory oestruses.

On the other hand, exogenous E_2 (5 mg) administration at the time of CIDR insertion in heifers (group II) resulted into a very high mean E_2 concentration of 111.25±39.81 pg/ml 24 h later, the decline was clearly visible by day 2. Interestingly on day 4, the mean E_2 levels were markedly lower in heifers than in cows. These differences became significant on days 6, 7, 8 and 9. It is our assumption that either the exogenous administration of oestrogen in these heifers or some other unknown factor(s) led to poor recruitment of follicles for growth and development. Since even the post-device withdrawal E_2 levels in these heifers were low (at best one and a half to twice the basal values), it may be assumed that optimum LH peak may not have resulted. It led to speculation that this could be responsible for anovulatory oestrus as indicated by only 20% conception rate at induced oestrus.

Use of E_2 along with P_4 releasing device was reported to have an inhibitory effect on the growth of follicles and emergence of next follicular wave may be delayed by about 4 days (Bo et al., 1995; Burke et al., 1999). Hanlon et al. (1996) used an injection of 0.5 mg OB 24 h after CIDR removal, probably to potentiate the endogenous E_2 peaks. A sub-sample of the same experimental animal population was subjected to RIA for LH peaks (Hanlon et al., 1997). The findings were interesting, as all treated animals had an LH peak compared to only in 55% of untreated heifers. The authors attributed the difference in conception rates of the E_2 treated and control animals to reduction in the variability of LH peak in E_2 treated group. E_2 treatment at 24 h post-device removal was also observed to bring about a better synchrony of oestrus, with large number of females being mated within 48 h of device removal.

It is quite likely that combining CIDR with Ovsynch may enhance the luteal synchrony as well as conception rate. Beneficial effect of keeping PRID or CIDR inserted in between 2 GnRH injections of Ovsynch or Cosynch protocols used for ovulation or oestrus synchronization in cows has been reported by many studies, as also evidenced by recent studies of supplementing the Ovsynch (Pursley et al., 2001; El-Zarkouny et al., 2004) and Cosynch protocols (Stevenson et al., 2003) with a CIDR insert resulting into increased serum P_4 values (at the time of $PGF_2\alpha$ administration), ovulation and pregnancy rates in cows compared to Ovsynch or Cosynch treatment alone. Alnimer and Lubbadeh. (2003) had also reported significantly higher first-service conception rates, greater synchrony of luteal function and decreased ‘day open’, after adding P_4 intravaginal device in timed AI oestrus synchronization protocol in dairy cattle.

Apparently, studies on large number of cows and heifers of Sahiwal breed are essential for a conclusive uniform protocol to be recommended.

The results of the present study indicate that even with a relatively low first-service conception, the overall pregnancy rates in CIDR treated anoestrus Sahiwal cows and heifers are highly satisfactory. Majority of the induced females exhibit cyclicity within 36 h post-device withdrawal, raising doubts about the success of fixed-time inseminations in synchronization protocols.

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