Effect of estrus synchronization on plasma progesterone profile and fertility response in postpartum suckled anestrous Kankrej cows

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ABSTRACT

The study was designed at evaluating early postpartum ovarian activity through plasma progesterone profile at 10 days interval up to 90 days, followed by induction of estrus in postpartum anestrous suckled Kankrej cows (n=18) using Ovsynch, CIDR and Ovsynch + CIDR treatment protocols with FTAI (6 cows in each protocol), keeping six normal cyclic cows as control. The plasma P₄ profile (ng/ml) varied in the range of 0.15±0.03 to 1.70±0.80 in treatment groups and 0.62 ±0.19 to 4.09±1.60 in control group up to 90 days postpartum. The mean P₄ concentration on the day of calving was low (<1 ng/ml) in all the groups, which gradually increased to reach peak levels by day 40 in cyclic control (4.09±1.60 ng/ml), Ovsynch (1.70±0.80 ng/ml) and CIDR (1.68±0.47 ng/ml) and by day 20 in Ovsynch + CIDR (1.09±0.23 ng/ml) groups. Most of the cows turned into subestrous or anestrous condition between days 40 and 90 postpartum. The estrus induction response in Ovsynch, CIDR and Ovsynch + CIDR groups initiated at day 90-92 postpartum was 66.66, 83.33 and 50.00 per cent, respectively. The conception rates at induced estrus were 16.66, 33.33 and 16.66 per cent, and overall of 3 cycles 33.33 (2/6), 50.00 (3/3) and 33.33 (2/6) per cent, respectively. In normal cyclic control group, the conception rates at first cycle and overall of 3 cycles were 33.33 and 50.00 per cent. The mean plasma P₄ (ng/ml) concentrations were significantly (P<0.05) higher on day 7 in Ovsynch (1.41 ±0.10), CIDR (4.92±0.83) and Ovsynch + CIDR (3.87±0.84) protocols as compared to their corresponding values on day 0 and day 9/10 (AI). The mean values of plasma P₄ from days 20 to 40 post-AI were higher in conceived cows than in non-conceived cows of CIDR and Ovsynch + CIDR groups, compared to the animals in Ovsynch and normal cyclic groups. It is opined that the use of different protocols, mainly CIDR, may serve as an effective tool for induction and synchronization of estrus and improvement of conception rate in postpartum anestrous suckled Kankrej cows.

Key words: Anestrous, Estrus synchronization protocols, Fertility rate, Postpartum period, Plasma progesterone, Suckled Kankrej cows.

INTRODUCTION

The profitable dairy farming is predominantly based on two major inter-related factors, viz., nutrition and reproduction. For economic dairy farming, dairy cows must calve regularly at every 12 to 14 months interval. Prolonged inter-calving period due to delay in onset of postpartum ovarian function is regarded as one of the major reproductive problems responsible for failure to maintain optimum reproductive efficiency, which in turn causes economic loss to the dairy farmers (Shamsuddin et al., 2006; Bhoraniya et al., 2012; Dhami et al., 2015). Kankrej cows have a majestic look and are a dual purpose zebu cattle breed of Gujarat, with prolonged postpartum subestrous and anestrous conditions. Suckling as well as the hot-humid climate of tropical countries like India enhances postpartum infertility problems in dairy animals. Fixed time AI (FTAI) protocols such as Ovsynch, CIDR and Cosynch have been developed to decrease reliance on detection of estrus in reproductive management programmes to establish sustainability in the dairy industry (Mohan Krishna et al., 2010; Keskin et al., 2011; Bhoraniya et al., 2012; Nakrani et al., 2014). The present study was therefore, focused to evaluate postpartum plasma progesterone profile till day 90 and to assess whether the reproductive performance of postpartum anestrous suckled Kankrej cows could be improved using different estrus induction/synchronization protocols.

MATERIALS AND METHODS

The postpartum suckled Kankrej cows (n=24) of the University farm were selected to monitor the plasma progesterone profile at 10 days interval from calving to day 90 postpartum together with reproductive status through gynaeco-clinical examinations. Completion of uterine involution and initiation of ovarian activity of each animal was assessed by palpation per rectum at five days interval from day 10 to 40 postpartum. The ovarian status was assessed by gynaeco-clinical examinations per-rectum at...
least twice at 10 days interval beginning at day 70 postpartum in cows not expressing behavioral estrus to confirm anestrous (n=18) and cyclic (n=6 control) cows. The anestrous animals were subjected to one of the three estrus induction/synchronization protocols viz., Ovsynch, CIDR and Ovsynch + CIDR (6 animals in each group) on day 90 postpartum with FTAI using good quality frozen thawed semen as explained by Bhoraniya et al. (2012).

**Ovsynch Protocol:** Anestrous cows having small smooth inactive ovaries (true anestrous) were administered with an intramuscular injection of 20 µg GnRH (Buserelin acetate, Receptal 5 ml, Intervet) on day 0, followed by an injection of 500 µg PGF$_2$α (Cyclix 2 ml, Intervet) on day 7 and a second GnRH injection of 20 µg on day 9. Fixed time AI (FTAI) was performed 22 hrs after second GnRH injection.

**CIDR Protocol:** Here the CIDR intra-vaginal device (1.38 g progesterone in elastic rubber molded over a nylon spine, Pfizer India Ltd.) was inserted with special applicator in true anestrous cows for 7 days and an i/m injection of 500 µg PGF$_2$α (Cyclix) was made on day 7 while removing the CIDR, and FTAI was done on day 9.

**Ovsynch + CIDR Protocol:** Another six postpartum anestrous cows were administered with an i/m injection of 20 µg GnRH (Receptal 5 ml) on day 0, accompanied by CIDR intra-vaginal insert. It was followed by an injection of 500 µg PGF$_2$α (Cyclix) on day 7, with removal of CIDR insert. A second i/m injection of GnRH 20 µg was administered on day 9 and FTAI was performed 22 hrs later.

**Control:** Six healthy normal cows exhibiting first estrus or having palpable follicle/CL on the ovaries within 3 months postpartum were included in cyclic control group. The cows not returned to estrus post-AI were palpated per rectum for confirmation of pregnancy on day 60 post-breeding. The cows returned to estrus were inseminated up to third cycle.

Blood samples were collected from all animals on the day of calving and at 10 days interval up to the initiation of estrus induction protocol in anestrous animals on day 90 postpartum, and on the day of AI from cyclic control animals. Further blood sampling was accomplished on day 0, day 7 (PGF$_2$α injection) and on the day of induced/ natural estrus (day 9/11, AI), and then on days 10, 20, 30 and 40 post-AI (Table 1). Blood samples collected in heparinized vacutainers through jugular vein puncture were immediately centrifuged at 3000 rpm for 15 minutes, and aspirated plasma samples were stored at -20°C until analyzed.

Plasma progesterone concentrations were estimated using RIA technique (Kubasic et al., 1984). The sensitivity of assay was 0.1 ng/ml. Intra- and inter-assay coefficients of variation were 5.4 and 9.1 per cent, respectively. Cross reactivity of the antibody with progesterone, 17α-dihydroprogesterone and 20α-hydroxyprogesterone was 100, 0.13 and 0.96 per cent, respectively. The differences in estrus induction response and conception rates of different estrus synchronization protocols were tested by using chi square test on arc sin transformed data. The progesterone profile was analyzed using an online package of SAS system (version 14.00) of statistical analysis.

**RESULTS AND DISCUSSION**

The results of present study obtained on plasma P$_4$ concentrations, oestrus response and conception rate are presented in Tables 1, 2 and Figure 1.

**Postpartum plasma progesterone profile:** The mean plasma P$_4$ concentrations from calving to 90 days postpartum in animals of cyclic control, Ovsynch, CIDR and Ovsynch + CIDR treatment groups varied from 0.62 ± 0.19 to 4.09 ± 1.60, 0.15 ± 0.03 to 1.70 ± 0.80, 0.40 ± 0.16 to 1.68 ± 0.47 and 0.39 ± 0.09 to 1.09 ± 0.23 ng/ml, respectively (Table 1). The mean plasma P$_4$ concentrations on the day of calving till 10 days postpartum were low (<1 ng/ml) in animals of all groups, which gradually increased to peak levels by day 20, 30 or 40 in animals of different groups (Table 1). These observations were associated with regression of pregnancy CL, initiation of ovarian activity and silent ovulations at varying intervals by day 40-50 postpartum in few animals of each group. Thus, majority of the cows had expressed cyclicity with significant change in plasma P$_4$ profile until 40-50 days postpartum, but then became anestrous with low P$_4$ levels (<1 ng/ml) due to peak milk yield and negative energy balance till treated with estrus synchronization protocols on day 90 postpartum.

In control group, all the animals expressed signs of estrus at least once between days 30 and 60 postpartum and then continued to cycle that may be the reason of rise in progesterone values from days 50 to 80 (Table 1, Fig. 1). Three animals of cyclic control group were inseminated on spontaneous estrus between days 70 and 90, of which two cows conceived and one returned to estrus on day 112 postpartum, the remaining three animals remained subestrous during the 90 days postpartum.

![Mean plasma P$_4$ concentration in conceived and non-conceived Kankrej cows from day of calving up to 90 days postpartum and from day of initiation of estrus synchronization treatments (day 90-95) up to day 40 post-AI.](image-url)
**Table 1:** Postpartum plasma progesterone profile (ng/ml) in different groups of Kankrej cows before and after initiation of various estrus synchronization protocols (Mean ± SE)

<table>
<thead>
<tr>
<th>Days postpartum</th>
<th>Group–I (Ovsynch)</th>
<th>Group–II (CIDR)</th>
<th>Group-III (Ovs+CIDR)</th>
<th>Group-IV (Cyclic control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0(^a)</td>
<td>0.96±0.13(^{bcd})</td>
<td>0.40±0.16(^e)</td>
<td>0.92±0.17(^d)</td>
<td>0.74±0.32(^c)</td>
</tr>
<tr>
<td>10</td>
<td>0.71±0.21(^{bcd})</td>
<td>1.22±0.20(^e)</td>
<td>0.63±0.14(^d)</td>
<td>0.62±0.19(^c)</td>
</tr>
<tr>
<td>20</td>
<td>1.17±0.52(^{bcd})</td>
<td>1.68±0.47(^{cde})</td>
<td>0.86±0.24(^d)</td>
<td>1.87±0.83(^{abc})</td>
</tr>
<tr>
<td>30</td>
<td>1.32±0.58(^{bcd})</td>
<td>0.99±0.73(^{de})</td>
<td>0.80±0.22(^d)</td>
<td>4.90±1.60(^{abc})</td>
</tr>
<tr>
<td>40</td>
<td>1.70±0.80(^{bcd})</td>
<td>1.35±0.34(^{de})</td>
<td>1.09±0.23(^d)</td>
<td>1.70±0.59(^{bc})</td>
</tr>
<tr>
<td>50</td>
<td>0.54±0.33(^{bcd})</td>
<td>1.04±0.27(^{de})</td>
<td>0.99±0.21(^d)</td>
<td>1.32±0.54(^{bc})</td>
</tr>
<tr>
<td>60</td>
<td>0.46±0.12(^{bcd})</td>
<td>0.68±0.32(^e)</td>
<td>0.43±0.11(^d)</td>
<td>3.20±1.05(^{abc})</td>
</tr>
<tr>
<td>70</td>
<td>0.15±0.03(^{bcd})</td>
<td>0.41±0.14(^e)</td>
<td>0.50±0.10(^d)</td>
<td>2.46±0.98(^{abc})</td>
</tr>
<tr>
<td>80</td>
<td>0.40±0.12(^{bcd})</td>
<td>0.48±0.08(^e)</td>
<td>0.39±0.09(^d)</td>
<td>3.84±1.48(^{abc})</td>
</tr>
<tr>
<td>90</td>
<td>0.54±0.14(^{bcd})</td>
<td>0.77±0.10(^e)</td>
<td>0.59±0.13(^d)</td>
<td>1.31±0.56(^{abc})</td>
</tr>
</tbody>
</table>

**Profile during and after estrus synchronization**

| D 0\(^a\) | 0.50±0.18\(^{bcd}\) | 1.01±0.05\(^{e}\) | 0.83±0.15\(^d\) | 4.54±1.20\(^{ab}\) |
| D 7\(*\) | 1.41±0.10\(^{bcd}\) | 4.92±0.83\(^{e}\) | 3.87±0.84\(^{d}\) | - |
| D 9/10 (AI) | 1.41±0.39\(^{bcd}\) | 0.74±0.13\(^e\) | 1.02±0.22\(^d\) | 3.11±0.97\(^{abc}\) |
| D 10 PAI | 3.09±1.80\(^{bcd}\) | 3.20±0.82\(^{abcd}\) | 1.60±0.30\(^{ad}\) | 3.69±0.94\(^{abc}\) |
| D 20 PAI | 5.17±1.36\(^e\) | 2.65±1.41\(^{bcd}\) | 2.12±1.13\(^{ad}\) | 3.22±0.74\(^{abc}\) |
| D 30 PAI | 2.95±1.50\(^{abc}\) | 4.11±1.88\(^{abcd}\) | 2.65±0.86\(^{abc}\) | 5.36±0.71\(^{abc}\) |
| D 40 PAI | 2.33±0.54\(^{bcd}\) | 3.71±1.47\(^{abc}\) | 2.98±1.16\(^{ab}\) | 4.71±1.62\(^{abc}\) |

**Means bearing uncommon superscripts within the column differ significantly (P<0.05)**

\(0^a\) = day of calving, * day of first treatment, ** day of PG injection. PAI = Post-AI.

The linear increasing trend of plasma P observed over the postpartum period with higher values in fertile/ conceived than infertile/ non-conceived animals in all the groups might be attributed to early resumption of follicular activity, ovulation and thereby conception in former group. The present findings are in accordance with those of Ammu et al. (2012a) and Naikoo et al. (2013) in Gir and Kankrej cows with similar weekly postpartum monitoring of plasma progesterone and ovarian changes. Patel et al. (2007) also monitored weekly plasma progesterone profile from the day of calving till 21 week in anestrous and subestrous HF cows with and without GnRH and PGF\(_{2}\alpha\) treatment at day 49 postpartum, respectively, and reported comparable findings.

The plasma progesterone profile is positively correlated with postpartum ovarian activity, particularly CL function in dairy animals, and helps to study ovarian changes.

**Response to estrus synchronization protocols:** The ovulatory estrus was induced in 66.66 (4/6) and 83.33 (5/6) per cent cows under Ovsynch and CIDR protocols, respectively, as confirmed by presence of CL on the ovary 12 days later associated with rise in plasma P\(_4\) level. Similar ovulatory response was reported with Ovsynch protocol by Deshmukh et al. (2009), Mohan Krishna et al. (2010) and Keskin et al. (2011) in cattle. The findings with CIDR are in harmony with the results of Khade et al. (2011) and Bhoraniya et al. (2012) in Gir heifers and Kankrej cows.

**Table 2:** Estrus induction response and conception rate in suckled Kankrej cows under different estrus induction/synchronization protocols

<table>
<thead>
<tr>
<th>Response to treatment</th>
<th>Ovsynch Protocol</th>
<th>CIDR Protocol</th>
<th>Ovsynch + CIDR Protocol</th>
<th>Untreated cyclic control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cows</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>No. of cows expressed behavioural estrus</td>
<td>4/6(66.66)</td>
<td>5/6(83.33)</td>
<td>3/6(50.00)</td>
<td>6/6(100.00)</td>
</tr>
<tr>
<td>No. of cows inseminated (FTAI/AI at observed estrus)</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>First service conception rate</td>
<td>1/6(16.66)</td>
<td>2/6(33.33)</td>
<td>1/6(16.66)</td>
<td>2/6(33.33)</td>
</tr>
<tr>
<td>Second service conception rate</td>
<td>Nil</td>
<td>1/4(25.00)</td>
<td>1/5(20.00)</td>
<td>1/5(25.00)</td>
</tr>
<tr>
<td>Third service conception rate</td>
<td>1/5(20.00)</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Overall conception rate in 3 cycles</td>
<td>2/6(33.33)</td>
<td>3/6(50.00)</td>
<td>2/6(33.33)</td>
<td>3/6(50.00)</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate percentage values.

The plasma progesterone profile is positively correlated with postpartum ovarian activity, particularly CL function in dairy animals, and helps to study ovarian changes.
The conception rates obtained at induced, second and third estrus with overall conception rate with Ovsynch treatment was 16.66, 00.00, 20.00 and 33.33 per cent (2/6), respectively. The corresponding conception rates for CIDR treatment were 33.33, 25.00, 00.00 and 50.00 (3/6) per cent and for normal cyclic control group 33.33, 25.00, 00.00 and 50.00 (3/6) per cent. The conception rates of CIDR were similar to that in the cyclic control group (Table 2).

In similar experiments with Ovsynch, Ghallab et al. (2009) reported conception rate of 40 per cent in HF cows, while Mohan Krishna et al. (2010) recorded it as 30 and 20 per cent in Sahiwal cows and heifers, respectively, which are almost similar to the present findings. On the contrary, higher conception rates of 50-56 per cent each with Ovsynch and CIDR protocols were recorded by Khade et al. (2011) and Bhoraniya et al. (2012) in Gir heifers and Kankrej cows, respectively. The conception results of the present study with CIDR agreed with the findings of Ammu et al. (2012) in Gir cows of the same farm. However, relatively lower conception rates of 26.00 to 42.74 per cent (Sathiamoorty and Kathirchelvan, 2010) and higher conception rates of 50 to 80 per cent (Patel et al., 2013; Nakrani et al., 2014; Dhami et al., 2015) have also been documented by others in crossbred cows. The prolonged exogenous progesterone priming from CIDR device might have caused negative feedback effect on hypothalamo-hypophyseal-gonadal axis and increased receptors for gonadotropins on the ovaries followed by rebound on its sudden withdrawal causing stimulated FSH secretion, folliculogenesis and ovulation.

The possible reasons for variation in results of different studies could be the stage of ovarian cycle at the beginning of the protocol, apart from variations in different environmental, management and genetic factors like nutritional status, parity, stage of lactation, suckling stimulus, season/climate, drug source, age, breed, and species of animal etc. We studied zebu-Kankrej cows which are known for poor reproductive efficiency as compared to exotic and crossbred animals etc. We studied zebu-Kankrej cows which are known for poor reproductive efficiency as compared to exotic and crossbred cows.

Among the six cows under Ovsynch plus CIDR combination protocol, 50 (3/6) per cent cows exhibited behavioural signs of estrus 48 hrs after PGF₂α injection. The remaining three cows became subestrous, with palpable ovarian structures. The conception rates at the induced estrus, second cycle and third cycle following this treatment were 16.66, 20.00 and 00.00 per cent, respectively, with an overall conception rate of 33.33 (2/6) per cent, which was similar to that of Ovsynch group and lower than in CIDR alone and control group (Table 2). No beneficial effect of combining Ovsynch with CIDR could be obtained in terms of induction of estrus and conception rate in the present study. However, higher conception rates (55.71 and 66.66 %) with this protocol were reported earlier by Fallah Rad and Ajam (2008) and Khade et al. (2011) in anestrous HF cows and Gir heifers, respectively.

The present findings suggest that the CIDR insert alone over Ovsynch or its combination improved synchrony of estrus occurrence and conception rate in anestrous suckler Kankrej cows.

**Plasma progesterone profile during and after synchronization treatments:** The plasma P₄ levels on day 90 postpartum and on the day of initiation of treatment indicated that animals of all three treatment groups were anestrous (Table 1, Fig. 1). The mean plasma progesterone levels in all the groups remained low on the day of AI, irrespective of whether they exhibited spontaneous or induced estrus after treatment.

In animals of Ovsynch group, there was a significant rise (P<0.05) in plasma progesterone level by day 7 after GnRH injection. This might be due to luteinization of some of the growing follicles and/ or ovulation of dominant follicle and formation of CL under the influence of GnRH simulating diestrum phase. In CIDR and CIDR + Ovsynch groups, the plasma P₄ levels showed a steep and significant increase (P<0.01) by day 7 in all animals. This was probably due to the exogenous supply of progesterone through CIDR insert.

The plasma P₄ levels were reduced by day 9 due to the effect of removal of CIDR and administration of PG injection on day 7 (Table 1). However, a slightly higher mean plasma P₄ noted on the day of AI in Ovsynch group could be due to two partially responsive (subestrous) and one non-responsive cow, whereas in Ovsynch + CIDR group, slight elevated mean level of plasma P₄ found could be due to partial treatment response (subestrous) in three cows. It may also be due to latent effect of exogenous progesterone given through CIDR. On day 10 post-AI the mean values of progesterone again increased significantly and remained high in pooled conceived group. However, in animals of pooled non-conceived group the values increased around day 10 post-AI, thereafter decreased drastically around day 20 post-AI and then showed a cyclic pattern as per second and third repeat cycle or conception (Fig. 1). This was because only one out of six cows in Ovsynch protocol conceived at induced estrus and one conceived at day 145 postpartum in third cycle. Two out of six cows treated with CIDR conceived at induced oestrus on day 100 and 105 postpartum and one conceived at day 124 postpartum in second cycle. One out of six cows treated with CIDR + Ovsynch conceived at induced oestrus on day 102 postpartum and one conceived on day 121 postpartum in second cycle (Table 2).

In control group, the mean plasma progesterone level increased significantly (P<0.05) on day 10 after Al and then remained at that elevated level till day 40 post-AI in conceived cows. Whereas, the level dropped around day 20 post-AI, and thereafter showed cyclic pattern up to day 40 post-AI in a non-conceived cow that returned to estrus.
Out of six cows, two conceived on first service on day 70 and 90 postpartum and one at second service on day 112 postpartum (Table 2).

The mean plasma progesterone levels obtained in the present study on the day of initiation of CIDR and Ovsynch treatments with significant rise on day 7 and sudden drop to basal levels on induced estrus within 48 h of PG injection corroborated with the earlier findings in Gir and crossbred cows (Ammu et al., 2012; Patel et al., 2013; Dhami et al., 2015) and anoestrous buffaloes (Nakrani et al., 2014; Savalia et al., 2014). Further, the statistically similar mean plasma progesterone concentrations observed on day 0, 7 and on day 9 (AI), but with higher values in the conceived than non-conceived cows on day 20 post-AI in all treatment protocols and in normal cyclic control group, and thus the pooled values of conceived and non-conceived groups (Fig. 1), closely corroborated with the earlier observations in anoestrous cows (Bhoraniya et al., 2012; Patel et al., 2013) and buffaloes (Naikoo et al., 2010; Nakrani et al., 2014; Savalia et al., 2014) under such protocols. The levels of plasma P_{21} on the day of beginning of treatment protocol helped delineate the reproductive and endocrine status of the animals and thereby predicting the possible response to the therapy. The variable plasma P_{21} recorded on day 21 post-AI in different groups was due to establishment of pregnancy and maintenance of CL function in few cows and return to next estrus at varying intervals on account of failure of conception or early embryonic mortality after day 17 in others. The findings of this study are also in line with the reports of Deshmukh et al. (2009) and Henricks et al. (2010).

CONCLUSIONS
The present study on plasma progesterone profile during postpartum period assisted in assessing postpartum ovarian activity, and in detecting silent estrus/ovulation in Kankrej cows. Induction and synchronization of estrus and improvement in conception rate is possible in postpartum anoestrous suckled zebu/Kankrej cows with the use of different protocols. Ovsynch protocol and application of CIDR resulted in better estrus expression and fertility as compared to their combination in infertile Kankrej cows. The linear increasing trend of plasma P_{21} observed over the postpartum period with higher values in fertile/conceived than infertile/non-conceived animals in all the groups might be attributed to early resumption of follicular activity and thereby conception in former group.

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REFERENCES


