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ORIGINAL RESEARCH

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

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Abstract The study was aimed at induction/synchronization of estrus in postpartum anestrous Kankrej cows of zebu cattle maintained at an organized farm. The study included use of different hormone protocols, viz., Ovsynch, CIDR (controlled internal drug release), Ovsynch plus CIDR, and Heatsynch with estimation of plasma progesterone on days 0, 7, 9/11 (artificial insemination—AI) and on day 20 post-AI following fixed time insemination. Thirty selected anestrous animals were divided into five equal groups (four treatment and one control), and the findings were compared with the normal cyclic control group of six cows. All the protocols were initiated in cows with postpartum anestrous period of more than 4 months, considering the day of first GnRH injection or CIDR insertion as day 0. The animals were bred by fixed time artificial insemination. Pregnancy was confirmed per rectum on day 60 post-AI in non-return cases. The conception rates at induced/first heat in Ovsynch, CIDR, Ovsynch + CIDR, and Heatsynch protocols were 33.33, 66.66, 50.00 and 16.67%, respectively. The corresponding overall conception rates of three cycles post-treatment were 50.00% (3/6), 100.00% (6/6), 66.66% (4/6), and 50.00% (3/6). In normal cyclic and anestrous control groups, the pooled pregnancy rates were 83.33% (5/6) and 16.67% (1/6), respectively. The pooled mean plasma progesterone (nanograms per milliliter) concentrations were significantly ($P<0.05$) higher on day 7 in Ovsynch (5.727±1.26), CIDR (4.37±0.66), Ovsynch plus CIDR (3.55±0.34), and Heatsynch (5.92±1.11) protocols as compared with their corresponding values obtained on days 0, 9/11 (AI), and on day 20 post-AI. In anestrous control group, the mean progesterone concentration at the beginning of experiment was 0.67±0.33 ng/ml, which was at par with values of all other groups. The overall plasma progesterone levels on the day of initiating treatment were low in all groups, with smooth small inactive ovaries palpated per rectum twice at 10 days interval, suggesting that most of the animals used in the study were in anestrous phase. Mean (±SE) values of plasma progesterone (nanograms per milliliter) on day 20 post-AI were higher in conceived cows than the non-conceived cows of all the groups, but differed significantly ($P<0.05$) only in normal cyclic group. These results suggest that use of different hormone protocols particularly Ovsynch, CIDR, and Ovsynch + CIDR may serve as an excellent tool for induction and synchronization of estrus and improvement of conception rate in postpartum anestrous Kankrej cows.

Keywords CIDR · Estrus synchronization · Fertility · Heatsynch · Kankrej cows · Ovsynch · Plasma progesterone

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Introduction

The postpartum anestrous in cattle has been identified as one of the prevalent problems affecting the reproductive efficiency, which is in turn a major source of economic loss to dairy farmers of rural areas. Suckling as well as the hot and humid climate of tropical countries like India usually aggravates the postpartum anestrous and other infertility problems in dairy animals. The livestock production in general and milk in particular plays an important role in national economy, and thus, dairying is one of the most important agricultural activities in India. To obtain an economically profitable calving interval, it is highly necessary to make dairy farmers aware of the economic losses due to postpartum anestrous, common in Kankrej cows. FTAI protocols such as Ovsynch and Heatsynch have been developed to decrease reliance on detection of estrus in reproductive management programs (Mohan Krishna et al. 2010; Keskin et al. 2011; Naikoo et al. 2011). Scientific investigations have shown that, for synchronization of ovulation, postpartum anestrous buffaloes subjected to Ovsynch plus CIDR protocol resulted in induction of estrus with acceptable ovulatory response and first service conception rate (Ravikumar et al. 2009; Naikoo et al. 2011). Heatsynch protocol has proved efficient for farms with a low estrus detection rate, as it is based on timed insemination (Stevenson et al. 2004; Mohan Krishna et al. 2009). The Kankrej breed of zebu cattle under Indian agro-ecosystem is known for prolonged postpartum anestrous and thereby extended calving interval requiring some remedial intervention to optimize it. The present study was therefore focused to evaluate and to improve the reproductive performance of postpartum anestrous Kankrej cows managed under Indian agro-ecosystem using different estrus induction/synchronization protocols based on estrus induction response, conception rate, and plasma progesterone profile.

Materials and methods

Thirty postpartum anestrous and six normal cyclic, lactating suckled Kankrej cows of the University Farm were utilized in this study. The anestrous animals had not shown any estrus signs for at least 4 months postpartum. Gyneco-clinical examinations performed per rectum at least twice at 10 days interval revealed normal genital tract with smooth small inactive ovaries. Uterine horns were found to be atonic. They were subjected to different estrus induction and synchronization protocols with FTAI using good quality frozen thawed semen. The cows not returned to estrus post-AI were palpated per rectum for confirmation of pregnancy on day 60 post-breeding. The animals selected were distributed in the following six groups.

Group I: Ovsynch protocol

The protocol included IM injection of 20 μg Buserelin acetate—GnRH analogue (Receptal®, 5 ml, Intervet International GmbH, Germany) followed by an injection of prostaglandin F_2 alpha-$PGF_2\alpha$ 500 μg (Cyclix®, 2 ml, Intervet International GmbH, Germany) on day 7 and a second injection of 20 μg GnRH (Receptal, 5 ml) on day 9. FTAI were performed twice at 12 and 24 h after second GnRH injection. The animals once inseminated were followed for pregnancy or estrus recurrence, if any. The cows returned to estrus were inseminated and followed for three cycles.

Group II: CIDR protocol

In this protocol, CIDR (1.38 g progesterone in elastic rubber molded over a nylon spine, Pfizer Animal Health, Berlin, Germany) was inserted intravaginally in six postpartum anestrous cows in combination with IM injection of 1.0 mg estradiol valerate (Progynon depot®, German Remedies). On the sixth day following insertion of CIDR, the cows were treated with a regular IM dose of $PGF_2\alpha$ 500 μg (Cyclix, 2 ml), and estradiol valerate 0.75 mg was given 24 h later. Double inseminations were performed between 48 and 72 h after $PGF_2\alpha$ injection.

Group III: Ovsynch plus CIDR protocol

CIDR plus Ovsynch protocol included insertion of CIDR device intravaginally for 7 days together with administration of Ovsynch protocol (Receptal, 5 ml; Cyclix, 2 ml and Receptal 5 ml on days 0, 7, and 9, respectively) followed by FTAI 12 and 24 h later.

Group-IV: Heatsynch protocol

Heatsynch protocol consisted of IM injection of 10 μg of GnRH analogue (Receptal; 2.5 ml) followed by IM injection of $PGF_2\alpha$ 500 μg (Cyclix, 2 ml) on day 7 and IM injection of estradiol benzoate 1 mg (Sigma Chemicals, USA) on day 8, followed by double inseminations at 48 and 60 h post-estradiol injection.

Group V: anestrous (untreated) control

Six anestrous Kankrej cows of similar nature were identified; blood samples were collected once and then kept under

observations for 2 months. Estrus expression, if any, was noted, and breeding was done accordingly.

Group VI: normal cyclic–fertile—control

Six healthy normal cows exhibiting first estrus within 3 months postpartum were included in this group. The cows were inseminated twice, 12 h apart, during natural estrus and were followed for pregnancy or repeat AI.

Blood samples were collected in heparinized vials from cows of all four treatment groups on day 0, i.e., the day of beginning of the experiment, day 7, day of estrus (first AI), and finally on day 20 post-AI following second FTAI 12 h later. In cows of control groups, blood samples were collected on day of estrus and again on day 20 post-insemination. The plasma samples were stored at −20°C till analysis. Plasma progesterone concentrations were estimated by employing standard radioimmunoassay technique of 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%, respectively. Cross-reactivity of the antibody with progesterone, 17α-dihydroprogesterone, and 20α-hydroxyprogesterone was 100%, 0.13%, and 0.96%, respectively.

The data on estrus induction and conception rates were analyzed using chi-square test and those of plasma progesterone profile by using completely randomized design and Student's paired *t* test (Snedecor and Cochran 1989).

Results and discussion

Improving reproductive efficiency through various managemental, breeding, and therapeutic means has been the primary goal of any economically viable dairy entrepreneur. In the present study, four different hormonal protocols were used to induce/synchronize estrus in anestrous Kankrej cows (six in each group) of the University farm, keeping six cows each as anestrous untreated control and normal cyclic–fertile control. The results obtained are presented in Tables 1 and 2 and Figs. 1 and 2.

Effect of Ovsynch protocol

All six (100%) cows of this group exhibited synchronized ovulatory estrus within stipulated time of 24 h with moderate to prominent estrus signs. The ovulation was confirmed by presence of palpable CL on the ovary 10–12 days later. The conception rates at the induced estrus and subsequent second and third cycles were 33.33%, 25.00%, and 0.00%, respectively, with an overall conception rate of 50.00% (3/6). The corresponding conception rates for normal cyclic control group were 50.00%, 66.66%, 00.00%, and 83.33%, but did not vary statistically from above values (Table 1). Among six anestrous control cows, only 16.67% (1/6) cows exhibited natural estrus after 28 days of initiation of the study, and it took two cycles to conceive. Among the three non-conceived cows of Ovsynch group, two cows showed repeat breeding and one again entered in to anestrous (Table 1).

The present findings of conception rates at induced estrus following Ovsynch protocol corroborated well with some reports (Stevenson et al. 2006; Keskin et al. 2011). Muneer et al. (2009) reported the estrus response of 100.00% but with 90.00% conception rate in crossbred cows. The estrus response of Kankrej cows under study was comparable with above report, but the conception rate was lower (33.33%), probably due to Kankrej being zebu breed with poor inherent breeding efficiency. Mohan Krishna et al. (2010) recorded estrus response of 60–70% in Sahiwal cows and heifers using same protocol with still lower conception rates after FTAI (20–30%). Naikoo et al. (2011) recorded first service and overall conception rates of 50.00% and 66.66%

Table 1 Effect of different hormone protocols on estrus induction/synchronization response and conception rate in postpartum anestrous Kankrej cows

Group	No. of cows	Estrus response	Conception rate at				Status of non-pregnant cows at 60 days post-AI	
			Induced estrus	Second cycle	Third cycle	Overall	Cyclic	Anestrous
Ovsynch protocol	6	100% (6/6)	33.33% (2/6)	25.00% (1/4)	0.00% (0/3)	50.00% (3/6)	2	1
CIDR protocol	6	100% (6/6)	66.66% (4/6)	50.00% (1/2)	100.00% (1/1)	100.00% (6/6)	0	0
Ovsynch + CIDR Protocol	6	100% (6/6)	50.00% (3/6)	0.00% (0/3)	33.33% (1/3)	66.66% (4/6)	2	0
Heatsynch protocol	6	100% (6/6)	16.66% (1/6)	40.00% (2/5)	0.00% (0/3)	50.00% (3/6)	1	2
Anestrous Control	6	16.67% (1/6)	0.00% (0/6)	16.67% (1/6)	0.00% (0/5)	16.67% (1/6)	0	5
Normal cyclic Control	6	100% (6/6)	50.00% (3/6)	66.66% (2/3)	0.00% (0/1)	83.33% (5/6)	0	1

Table 2 Plasma progesterone concentration (nanograms per milliliter) on different days of treatment/AI in Kankrej cows under various estrus induction/synchronization protocols

Estrus synchronization protocols	Status at induced estrus	No.	Days from treatment/AI			
			D-0	D-7	D-9/11 (AI)	D-20
Ovsynch	Conceived	2	0.35±0.25	5.65±2.35	0.23±0.13	3.20±0.10
	Non-conceived	4	1.72±0.75	5.75±1.75	0.70±0.29	2.04±1.07
CIDR	Conceived	4	0.35±0.07	3.53±0.53	0.12±0.17	3.45±0.17
	Non-conceived	2	0.70±0.10	6.05±0.75	0.71±0.39	1.60±0.50
Ovsynch + CIDR	Conceived	3	0.87±0.54	4.07±0.29	0.66±0.23	3.70±0.35
	Non-conceived	3	1.20±0.46	3.03±0.49	1.18±0.32	2.20±0.89
Heatsynch	Conceived	1	0.33	8.00	0.17	2.60
	Non-conceived	5	0.63±0.38	5.50±1.26	0.57±0.32	1.94±0.81
Anestrous control	Conceived	–	–	–	–	–
	Non-conceived	6	0.67±0.33	–	–	–
Normal cyclic control	Conceived	3	–	–	0.60±0.06	3.53*±0.56
	Non-conceived	3	–	–	0.80±0.29	1.03±0.44

Day 0, day of starting the treatment

Day 7, administration of $PGF_2\alpha$

Day 9/11, fixed time artificial insemination

Day 20, 20 days post-AI following second fixed time insemination

*$P<0.05$ between conceived and non-conceived subgroups

in anestrous buffaloes. The present findings suggest that application of Ovsynch protocol is quite useful in initiating cyclicity in acyclic Kankrej cows.

Effect of CIDR protocol

There was cent per cent ovulatory estrus induction and synchronization with prominent estrus signs in most of the animals of CIDR group. The conception rates through AI at the induced, second, and third cycle were 66.66%, 50.00%, and 100.00%, respectively, with an overall conception rate of 100.00% (6/6). These results of CIDR protocol were at par with the normal cyclic/fertile group (Table 1). Present results with CIDR were at par or superior as compared with those reported earlier in the literature. These higher overall and first service conception rates together with good visual estrus detection rate in this group might be attributed to prolonged exogenous progesterone priming from CIDR device with negative feedback effect on hypothalamo–hypophyseal–gonadal axis and increased receptors for gonadotropins on the ovaries followed by rebound on its sudden withdrawal, thus stimulating FSH secretion, folliculogenesis, and ovulation, and also to the use of estradiol valerate on the day of removal of CIDR that exaggerated the estrus signs.

Sathiamoorthy and Kathirchelvan (2010) and Naikoo et al. (2011) reported the pregnancy rates of 42.74% to 66% with CIDR protocol in cows and anestrous buffaloes, respectively. Chenault et al. (2003) also reported much lower values with non-significant difference in conception rate and pregnancy rate of lactating HF cows for CIDR-treated (26.7%, 12.2%) and control (30.9%, 11.1%) groups and

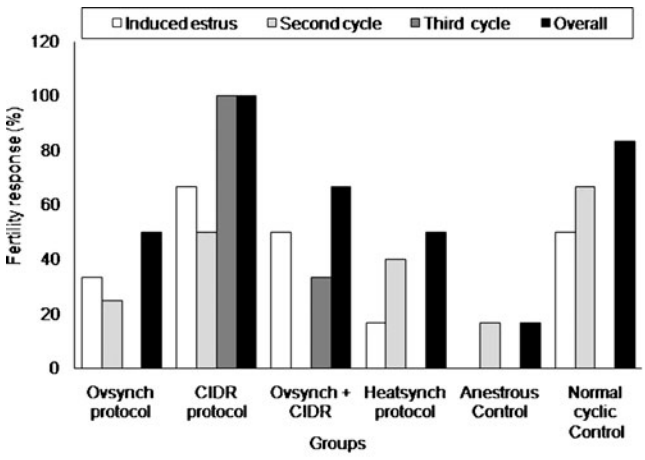

Fig. 1 Fertility response (conception rate) of anestrous Kankrej cows to different estrus induction and synchronization protocols in relation to control groups

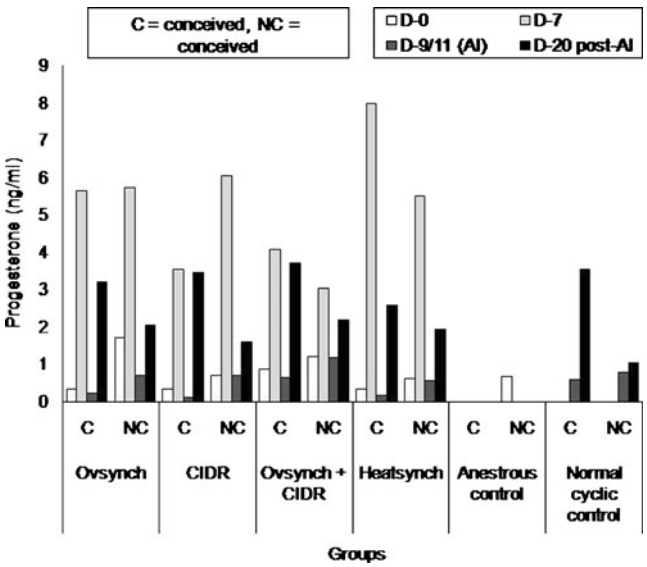

Fig. 2 Mean plasma P_4 concentration in conceived and non-conceived Kankrej cows of various estrus synchronization protocols on different days of treatment

concluded that CIDR inserts improved synchrony of returns to estrus, slightly reduced pregnancy rate to initial AI, but did not affect conception rate or pregnancy rate to AI during the resynchrony period. The explanation for better estrus response and conception rate in this group is establishment of proper endocrine harmony, progesterone priming, FTAI twice after last estradiol valerate injection, favorable season, good body condition of the animals, and better management at the farm. The results indicate that CIDR protocol is the best among all protocols tested to induce ovulatory estrus and conception in postpartum anestrous zebu cows.

Effect of Ovsynch plus CIDR protocol

A combined Ovsynch–CIDR protocol used on six postpartum anestrous Kankrej cows induced and synchronized estrus within 24 h of second GnRH injection in all the six animals with prominent, moderate, and weak estrus signs in three, two, and one cows, respectively. The conception rates at induced (FTAI), second, and third estrus/cycle were 50.00%, 0.00%, and 33.33%, respectively, with an overall conception rate of 66.66% (4/6). Two unconceived cows of this group remained cyclic rather than repeat breeder after 60 days of post-AI follow-up (Table 1). The reason for higher first service conception rate in this group could be the synergistic effect produced by Ovsynch and CIDR combination, FTAI at two times, i.e., 12 and 18 h after last GnRH injection and again, favorable season, good body condition of the animals, and better management at the farm.

Naikoo et al. (2011) recorded slightly higher first service and overall conception rates (66.66% and 83.33%) in acyclic buffaloes during breeding season. The first service conception rate achieved with Ovsynch and Ovsynch + CIDR protocols in the present study coincided with the report of Ravikumar et al. (2009), who obtained ovulatory response and first service conception rates of 83.33% and 33.33% with Ovsynch and 100.00% and 41.66% with Ovsynch plus CIDR groups, respectively. Stevenson et al. (2006) also recorded overall conception rate of 40% and 50% at day 28, and 33% and 38% at day 46 after TAI in Ovsynch and Ovsynch + CIDR protocols, which is in line with but poorer than the results obtained with the two protocols in the present study. These differences could be due to variation in number and breed of animals, nutritional status, and season. Our results suggest that application of Ovsynch plus CIDR protocol is useful in initiating cyclicity in acyclic animals in comparison to Ovsynch alone, and it can serve as one of the better means of estrus/ovulation synchronization and conception in postpartum anestrous Kankrej cows.

Effect of Heatsynch protocol

The Heatsynch protocol induced estrus in all six anestrous animals with prominent and moderate estrus signs in four and two cows, respectively. The conception rates following AI at induced, second, and third estrus were 16.66%, 40.00%, and 0.00%, respectively, with an overall conception rate and pregnancy rate of 50.00% (3/6) each (Table 1). Though homosexual behavior, vaginal mucus discharge and uterine tonicity were much prominent; the fertility results of this protocol were much poorer as compared with cyclic control group or any of the other protocols tried. This could be due to reduced doses of GnRH (one half) and potent estradiol benzoate used in place of estradiol valerate in this group. Moreover, two cows turned anestrous again after 60 days of treatment with Heatsynch (Table 1).

Brusveen et al. (2009) reported that supplementation with estrogen hormone–estradiol-17β (E_2) increased the expression of estrus (84.4% vs. 37.2%), but had no effect on overall fertility and even tended to have a negative effect on fertility in cows that ovulated to the second GnRH injection during the Ovsynch protocol (control=51.5% vs. E_2=44.0%). Leyva and Mellado (2009) obtained identical number of cows with intense estrus and conception rate for Ovsynch (n=20) and Heatsynch (n=38) protocols among repeat breeding HF cows that were induced to lactation. Mohan Krishna et al. (2009) documented an increased percentage of conceptions to total number of estruses in Heatsynch-treated buffaloes over the control group both in summer (26% vs 18%) and winter (40% vs 30%) seasons. The conception rates obtained in cows under study were higher than their observations. This difference might be due to high incidence of silent heat in case of buffaloes as compared with cows.

Overall comparison

The comparative success rate of four estrus induction/synchronization protocols, viz., Ovsynch, CIDR, Ovsynch + CIDR, and Heatsynch used on six anestrous Kankrej cows each (Table 1, Fig. 1) revealed that though cent per cent cows expressed synchronized estrus, the first service conception rate varied greatly between these protocols, and the values were 33.33%, 66.66%, 50.00%, and 16.66%, respectively, as compared with 50.00% first service conception rate in normal cyclic–control-cows, CIDR protocol being superior over Heatsynch protocol. The overall conception rates of three cycles over the 60-day period following induced estrus with Ovsynch, CIDR, Ovsynch + CIDR, and Heatsynch protocols were 50.00%, 100.00%, 66.66%, and 50.00% as against 83.33% in normal cyclic and 16.67% in anestrous control groups. Stevenson et al. (2004) compared

Ovsynch, Ovsynch + CIDR, Heatsynch, and Heatsynch + CIDR protocols to induce ovulatory estrus in lactating cows and concluded that substituting estradiol cypionate for second GnRH injection of Ovsynch in Heatsynch protocol resulted in more cows in estrus but slightly fewer ovulating.

Plasma progesterone profile

Out of 30 cows that were induced to estrus or expressed natural estrus and were inseminated among the six groups, 13 cows conceived and 17 cows returned to next estrus at different time intervals. The number of cows conceived at induced estrus varied from one to four (16.66% to 66.66%) in different groups. The plasma progesterone levels were low at the initiation of treatment in almost all the animals, but increased by three- to tenfold on day 7 post-treatment due to luteinization of secondary follicles or ovulation of dominant follicles and formation of accessory corpora lutea under the effect of GnRH, or direct exogenous source of progesterone (CIDR). The levels then decreased drastically around the day of estrus/AI due to luteolysis induced by $PGF_2\alpha$ injection or withdrawal of CIDR insert. Again, increased plasma progesterone levels were detected on day 20 post-insemination in conceived cows but were lower in non-conceived cows. Animals which failed to conceive had relatively high plasma P_4 at the time of AI (Table 2).

Statistically, the pooled mean plasma progesterone (nanograms per milliliter) concentrations were significantly ($P<0.05$) higher on day 7 in Ovsynch (5.727±1.26), CIDR (4.37±0.66), Ovsynch plus CIDR (3.55±0.34), and Heatsynch (5.92±1.11) protocols as compared with their corresponding values obtained on day 0 and on day 9/11 (AI). In anestrous control group, the mean progesterone concentration at the beginning of study was 0.67±0.33 ng/ml, which was at par with values of all other groups. The overall plasma progesterone levels on the day of initiation of treatment were the lowest in all groups, in addition to smooth small inactive ovaries palpated per rectum twice at 10 days interval, indicating that most of the animals used in the study were in anestrous phase when treatment was initiated. Mean (±SE) values of plasma progesterone (nanograms per milliliter) on day 20 post-AI were higher in conceived cows than the non-conceived cows in all the groups, but differed significantly ($P<0.05$) only in case of normal cyclic group (Table 2, Fig. 2). Naikoo et al. (2011) reported similar results in buffaloes. Siqueira et al. (2009) recorded the average P_4 levels in pregnant and non-pregnant crossbred cows and heifers as 5.2 ± 5.0 and 3.8 ± 2.4 ng/ml ($P=0.02$), respectively, on day 23 post-embryo transfer.

Melendez et al. (2006) speculated that an explanation for improved fertility and greater progesterone in the Presynch + Ovsynch + CIDR cows over those cows not receiving a CIDR insert might have occurred because the progesterone supplementation may induce more synchronized ovulation and normal luteal phases, which is consistent with finding from Stevenson et al. (2006). One possible explanation for higher conception rate in CIDR-treated cows is the progesterone via the CIDR that helped the cows to become slightly more fertile. Progesterone could possibly be affecting the oocyte quality or the environment in the uterus and its secretions. Although the specific action(s) of progesterone to improve fertility in timed AI programmes remains poorly defined, results of the current study and others (Melendez et al. 2006; Stevenson et al. 2006) indicate a rather consistent benefit. It is reasonable to speculate that exogenous progesterone may in some way enhance the quality of the uterine environment and improve the likelihood of successful pregnancy.

Conclusions

It was concluded that induction of cyclicity, estrus synchronization, and improvement in conception is possible with the use of different hormone protocols, viz., Ovsynch, CIDR, Ovsynch + CIDR, and Heatsynch in anestrous Kankrej cows. Although estrus induction was cent per cent with all hormone protocols, their relative efficacy varied. CIDR alone provided excellent fertility with conception rate up to cent per cent, whereas Ovsynch and Ovsynch + CIDR showed good fertility and Heatsynch yielded relatively poor conception rate, which was attributed to reduced dose of GnRH and high potency of estradiol benzoate used. Plasma progesterone profile helped in confirmation of ovarian status at the beginning of treatment and even conception in inseminated animals. Thus, to maintain a high degree of breeding efficiency among lactating Kankrej cattle herds of the tropical regions like India, use of advanced reproductive management strategies like estrus induction/synchronization appears to be a valuable tool.

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