INFLUENCE OF ESTRUS SYNCHRONIZATION PROTOCOLS ON FERTILITY PLASMA PROGESTERONE AND BIOCHEMICAL CONSTITUENTS IN KANKREJ COWS*

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ABSTRACT

The study was aimed to know the influence of different estrus synchronization protocols on plasma progesterone profile, certain biochemical constituents and fertility in 30 postpartum anoestrus Kankrej cows. The study included use of Ovsynch, CIDR, Ovsynch plus CIDR and Heatsynch protocols on 24 anoestrus cows (6 in each group) with blood sampling on day 0, 7, 9/11(AI) and on day 20 post-AI. One group each of 6 anoestrus and 6 normal cyclic cows was kept as untreated control and normal fertile control. There was 100 per cent estrus induction response in all the treatment protocols. These animals were bred by fixed-time AI. Pregnancy was confirmed per rectum on day 60 post-AI. The conception rates at induced estrus in Ovsynch, CIDR, Ovsynch + CIDR and Heatsynch protocol were 33.33, 66.66, 50.00 and 16.67 per cent, respectively, as against 50.00 per cent in normal cyclic group, while none of the anoestrus control animals expressed estrus till 32 days of follow-up period. The mean plasma progesterone concentrations were significantly higher on day 7 (5.727±1.26, 4.37± 0.66, 3.55±0.34 and 5.92±1.11 ng/ml, respectively) as compared to the corresponding values on day 0, 9/11 (AI) and on day 20 post-AI in all the 4 protocols. The overall mean plasma progesterone levels on the day of initiating treatment were low in all groups, rose following treatment and dropped to basal level on the day of induced estrus (AI) and again rose significantly by day 20 post-AI in conceived as compared to non-conceived cows. Biochemical investigations revealed that plasma total protein, total cholesterol, calcium and inorganic phosphorus levels were within normal physiological limits with overall means of 5.93±0.09 g/dl, 162.69±3.56 mg/dl, 9.76±0.12 mg/dl and 5.11±0.14 mg/dl, respectively. Their levels neither varied significantly between sampling days of different protocols nor between protocols on any of the days/periods studied. The results showed that neither the reproductive status (cyclic/anoestrus) nor estrus synchronization protocols influenced the blood biochemical constituents studied, though they had significant effect on plasma progesterone profile and fertility of animals.

Key words: Estrus synchronization protocols, Plasma progesterone, Metabolites, Kankrej cows

INTRODUCTION

The livestock production in general and milk in particular plays an important role in our national economy. One of the factors decreasing reproductive efficiency in high-producing herds is the decrease in expression and detection of estrus (Nebel *et al.*, 1997). Progesterone level is directly related with CL function, estrous cycle control and fertility of the cow. It is responsible for stimulation of cyclicity, follicular development and for continuation of pregnancy. In normal cyclic animals, cholesterol and progesterone levels are expected to be low and high, respectively, during diestrus stage and subsequently should alter during estrus stage (Singh *et al.*, 1983; Singh and Singh, 2006). Anoestrus cases however carry irregular levels

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of them which create uncertainty in exhibition of next estrus and the stage is continued for months together unless disrupted by endogenous or exogenous hormones. Response to any therapy is generally better in animals with optimal nutritional status. Zebu breeds of cattle are known for prolonged postpartum anoestrus and thereby extended calving interval making them uneconomic as dairy animals. Hence, this study was carried out to evaluate the effect of different estrus synchronization protocols on estrus induction/fertility rate, and plasma progesterone and biochemical constituents in postpartum anoestrus Kankrej cows.

MATERIALS AND METHODS

Thirty postpartum anoestrus and 6 normal cyclic Kankrej cows of Livestock Research Station of the University at Anand were utilized in this study. The anoestrus animals selected had not shown any estrus signs for at least four months postpartum and later were confirmed by per-rectal examinations twice at 10 days interval. They were distributed in five equal groups, viz., Ovsynch, CIDR, Ovsynch + CIDR, Heatsynch, and anoestrus control and the results were compared with a group of normal cyclic control. Anoestrus animals were clinically treated and bred by FTAI as per standard protocols (Bhoraniya *et al.,* 2012). The cows not returned to estrus post-AI were palpated per rectum for pregnancy on day 60 post-AI.

Blood samples were collected in heparinized vials from cows of all four treatment groups on day zero, i.e. the day of beginning of the treatment, day 7 (day of PG injection/ CIDR removal), day of induced estrus/AI and finally on day 20 post-insemination, while in normal cyclic cows 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 per cent, respectively. Plasma total protein, total cholesterol, calcium and inorganic phosphorus levels were estimated by using standard procedures and kits procured from Coral Clinical System, Goa on an auto-analyzer. The data on estrus induction and conception rates were analyzed by chi-square test and those of plasma progesterone profile and biochemical constituents by using CRD and 't' test (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

In anoestrus control group only one (16.67 %) cow expressed natural estrus with moderate signs after 32 days, while in normal cyclic control group all the six (100 %) cows showed prominent estrus signs. Among all treatment protocols, 100 per cent cows expressed estrus but the intensity varied. Of the animals that underwent the CIDR protocol, 4, 1 and 1 cows showed prominent, moderate and weak estrus signs, respectively. Similarly in Ovsynch + CIDR protocol 3, 2 and 1 cows, in Ovsynch protocol 2, 3 and 1 cows and in Heatsynch protocol 4, 2 and 0 cows showed prominent, moderate and weak estrus signs, respectively. In other words, 33.33, 66.66, 50.00 and 66.66 per cent of cows under Ovsynch, CIDR, Ovsynch + CIDR and Heatsynch protocol expressed prominent heat symptoms, respectively, like natural ones. Greater number of cows showing prominent estrus with CIDR and Heatsynch protocol could be due to administration of estradiol valerate and estradiol benzoate, respectively, on the last day of these two protocols. Similar results have also been documented earlier by other workers (Pancarci *et al.*, 2002; Stevenson *et al.,* 2004; Naikoo, 2009; Ravikumar *et al.*, 2009)

The comparison of 4 estrus synchronization protocols revealed that though 100 per cent cows expressed synchronized estrus, the conception rate at induced estrus varied greatly between protocols, and the values were 33.33, 66.66, 50.00 and 16.66 per cent, respectively for Ovsynch, CIDR, Ovsynch + CIDR and Heatsynch as compared to 50.00 per cent first service conception rate in normal cyclic cows, the CIDR protocol being superior over Heatsynch protocol. Pancarci *et al.* (2002) reported identical pregnancy rates (37.1±5.8 and 35.1±5.0 % in Florida and 28.2±3.6 and 29.0±3.5 % in Texas) after TAI following Ovsynch and

Heatsynch protocols in lactating dairy cows. Asokan *et al.* (2005) recorded ovulatory response of 54.54 and 68.63 per cent, and first service conception rate of 00.00 and 18.18 per cent, respectively, with Ovsynch and Ovsynch + CIDR protocols in postpartum anoestrus buffaloes. Stevenson *et al.* (2004) compared Ovsynch, Ovsynch + CIDR, Heatsynch and Heatsynch + CIDR protocol to induce ovulatory estrus in lactating cows. Second injection of GnRH in Ovsynch was replaced by estadiol cypionate in Heatsynch protocol. Incidence of estrus was less after GnRH (54%) than after ECP (87%), but more GnRH-treated cows ovulated (100 vs. 86%). Pre-treatment with CIDR had no effects on incidence of estrus or ovulation.

Overall, out of 30 inseminated cows 13 cows conceived at the induced/natural estrus, while 17 cows returned to next estrus at different time intervals. The percentage of cows conceived at induced estrus varied from 16.67 to 66.66 in different groups. The P_4 profile at the initiation of treatment was low/basal in most of the groups/ animals, suggesting their anoestrus status (Table 1). Although one cow in Ovsynch and 2 in Ovsynch + CIDR protocol had higher plasma progesterone level initially suggesting unapparent cyclicity. The plasma progesterone concentrations were significantly higher on day 7 as compared to corresponding values on day 0, 9/11 (AI) and on day 20 post-AI in all the 4 treatment protocols. Similar results were reported by Naikoo (2009) in anoestrus buffaloes. Relatively higher mean values of plasma progesterone on day 20 post-AI in some of the non-conceived cows could be due to abnormal nature of cycle, delayed embryonic mortality or unexplained endocrine alterations in them.

Significantly higher plasma progesterone levels recorded on day 7 in CIDR and Ovsynch plus CIDR groups might be due to the continuous release of the exogenous progesterone from the progesterone molded elastic coil inserted in the anterior vagina of the cows, while in the Ovsynch and Heatsynch protocol the higher level of the progesterone on the day 7 might be due to luteinization of some of the growing follicles or development of accessory CLs from ovulation of dominant follicles under the influence of first GnRH injection (Gumen *et al.*, 2003) simulating diestrus phase. Raghorte *et al.* (2009[a,b]) found significant variation in progesterone profile on different days following Ovsynch protocol with higher average P_4 levels in pregnant heifers and postpartum buffaloes on 21st day post-breeding. Muhammad *et al.* (2000) reported the plasma progesterone concentrations post-insemination as 2.30 - 4.00 and 0.10 - 2.20 ng/ml in pregnant and non-pregnant HF cows, respectively, which is similar to the present finding in Kankrej cows.

The overall pooled mean values of total protein and cholesterol obtained in anoestrus and normal cyclic cows under study were 5.89±0.23 vs 6.20±0.20 g/dl and 165.62±4.45 vs 183.18±8.20 mg/dl, respectively. The mean plasma protein and cholesterol concentrations neither varied significantly between sampling days of any protocol nor between protocols on any of the days/ periods studied. The values and trend between conceived and non-conceived cows were also inconsistent among different groups.

These findings on total protein are in close agreement with Dhami *et al.* (2007), who reported serum protein level as 6.80±0.10 g % in anoestrus cows. Kumar *et al.* (2009) also did not find variation in plasma total protein of conceived and non-conceived cows of either normal cyclic or repeat breeding groups. Similarly, Kulkarni (1990) reported non-significant alternation in the serum total protein level before and after treatment in anoestrus cows.

In the opinion of Henricks *et al.* (1971), the highest adrenal cholesterol value occurred at estrus, when female are under estrogen dominance eventually facing a decline later, when the progesterone phase sets in. Singh *et al.* (1983) observed that high level of cholesterol increased the estrogen level resulting in manifestation of heat, as cholesterol is the precursor of steroid hormones, but the results obtained in the study contradict this statement. Kumar *et al.* (2009) recorded higher plasma total cholesterol concentration in normal cyclic than the repeat breeder cows, though there was no significant difference in the levels between conceived

and non-conceived groups. On another hand, Jain and Pandita (1995) and Singh and Singh (2006) observed the total serum cholesterol level to be significantly higher at induced estrus, which differ from the results obtained in the present study in Kankrej cows. This might be due to the breed difference, assay technique and plane of nutrition.

The pooled values of plasma calcium and inorganic phosphorus in anoestrus and normal cyclic group were 9.67±0.23 vs 8.01±0.79 mg/dl and 5.12±0.23 vs 5.20±0.41 mg/dl, respectively. The values of both calcium and inorganic phosphorus noted on different days of the treatment with Ovsynch, CIDR, Ovsynch + CIDR and Heatsynch protocols neither varied significantly between protocols nor between days within the protocol. The values and trend between conceived and non-conceived cows were also inconsistent between different groups.

These findings to some extent corroborated with those of Goswami (1993), who also did not observe significant difference in the levels of serum calcium and inorganic phosphorus before and after non-hormonal treatment of anoestrus Red Kandhari cows. However, Kumar *et al.* (2009) reported significantly higher serum calcium and lower inorganic phosphorus in normal cyclic than the repeat breeding crossbred cows, while Singh *et al.* (2007) reported significantly lower concentration of serum calcium and higher phosphorus in cyclic as compared to anoestrus Hariana cows. The phosphorus was significantly higher in conceived than non-conceived group (Kumar *et al.*, 2009).

The results in general showed that neither the reproductive status nor the estrus synchronization protocols influenced the blood biochemical constituents studied, though they had significant effect on plasma progesterone profile and fertility of animals.

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