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Influence of Total Protein, Serum Glucose and Haemoglobin During Induced Estrus on Pregnancy Rate in Anestrus Crossbred Jersey Cows

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ABSTRACT

The present study was conducted to assess the influence of total protein, serum glucose and haemoglobin levels during induced estrus on pregnancy rate in 30 postpartum anestrus crossbred dairy cows. The cows were treated with progesterone (CIDR) based estrus induction protocol with double fixed timed inseminations at 12 and 24 h after injection of last GnRH. Estrus induction response was 100% with majority of the cows (80%) showing intermediate intensity of estrus. On the day of first AI, blood was collected from all the animals for the estimation of glucose, total protein and haemoglobin. The mean time to onset of estrus from the time of removal of CIDR was 44.85 ± 7.23 h. The overall conception rate in the present study was 30 % (9/30). In the present experiment, the mean glucose, total protein and haemoglobin at the time of estrus were 43.81 ± 1.14 mg/dl, 7.78 ± 0.24 g/dl and 8.68 ± 0.22 g/dl respectively. The mean glucose, total protein and haemoglobin levels in the anestrus cows which were included in the study are lower than the normal range. There is no significant different in the biochemical parameters among the cows showing weak, intermediate and intense intensity of estrus. Similarly, there is no significant difference in the biochemical parameters between the cows that conceived and cows not has not conceived. The finding in the present study shows that poor nutritional status may be the cause for the anestrus condition of the animal under field conditions.

Keywords: Postpartum anestrus, Crossbred cows, Estrus induction, CIDR, Biochemical parameters.

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INTRODUCTION

Early resumption of estrous cycle following calving is important for high reproductive efficiency in dairy cattle (Rhodes *et al.*, 2003). Delay in resumption of ovarian activity following calving adversely affects the economics of the dairy farming (Pasha *et al.*, 2021). Following calving, deviation in follicular growth, selection of a dominant follicle, follicular maturation,

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ovulation, and followed by luteolysis, result in restoration of cyclical ovarian activity. Failure of any of these events prolongs postpartum anestrus in dairy cows (Peter *et al*., 2009). Postpartum anestrus is affected by several factors such as general health, body condition score, plan of nutrition, milk yield, suckling, parity, season, breed, dystocia, presence of bull, uterine palpation, carry over effects from previous calving (Short *et al*., 1990). Normal blood levels of various biochemical constituents are indispensable for normal function of various systems of body including reproductive system. Changes in various biochemical constituents have been blamed for reproductive failures especially prolonged postpartum anestrus. In the recent years considerable attention has been focused on biochemical constituents of blood that have greater diagnostic value in evaluating the physiological status as well as in the clinical practice to improve postpartum fertility in female bovines (Butler, 2003). Several estrous synchronization protocols using different hormone are being applied to postpartum anestrus dairy cows, but the results are varying (Mehrajuddin *et al.*, 2016; Yániz *et al*., 2004). One of the major reasons for inconsistent response to estrus induction treatment is nutritional status of the animals (Chaudhari *et al*., 2012). Hence, the present research work was designed to the study the influence of total protein, serum glucose, and haemoglobin during induced estrus on conception rate following fixed timed AI in postpartum anestrus crossbred Jersey cows.

MATERIALS AND METHODS

The present study was conducted on thirty anestrus cows reared by rural dairy farmers of Puducherry region with a history of not showing signs of estrus even after 45 days postpartum. All the thirty cows were without any palpable uterine disorders and not having CL on the ovaries on two successive per rectal examinations at 10 days interval. All the animals were maintained under uniform management conditions, viz, grazing, substituted by stall feeding of fodder and concentrates.

All the cows were treated with Progesterone based estrus induction protocol. This protocol includes insertion of CIDR (EAZI-BREED CIDR, Manufactured by DEC International Ltd; Hamilton, New Zealand and marketed by Pfizer Animal Health, Mumbai, containing 1.38g Progesterone) intravaginally on day 0 with or without GnRH (15 cows received GnRH on Day 0), injection PGF2α (Pragma (Intas Pharmaceuticals Ltd; Ahmedabad, containing Cloprostenol @ 250 mcg/ml) 2 ml, intramuscularly on day 6 and removal of CIDR on day 7, injection GnRH (Ovulanta, Vet Mankind, New Delhi, containing Buserelin acetate @ 4 mcg/ml) 2.5 ml, intramuscularly on day 9 and double fixed time inseminations with frozen semen at 12 and 24 h after the last injection of GnRH.

Even through AI has been done on fixed time following CIDR removal, the experimental animals were observed for the signs of estrus at least two to three times a day for a minimum of 30 min during early morning, noon and early evening (Yizengaw *et al*., 2017) so as to record the intensity of estrus and onset of estrus following estrus induction protocol. Based on the different parameters like behavioural changes, physiological changes and gynaecological observations, the intensity of estrus was classified as intense, intermediate and weak estrus by using estrus score card (Krishnakumar, 2001). The percentage of animals showing estrus were estimated as the number cows exhibited the behavioural signs of estrus after removal of CIDR. Onset of estrus was calculated in hours from the time of removal of CIDR to the time of first appearance of estrus signs. Blood samples were collected from jugular vein with the help of 18-gauge needle from all experimental animals on day 9 and at the time of first AI into heparinized and non-heparinized tubes and placed in ice box immediately. The heparinized blood (whole blood) was used for estimating haemoglobin and glucose within one hour of collection. The non-heparinized blood was allowed to clot at room temperature and the serum was separated by centrifuging at 3000 rpm for 10 min. Serum was stored at -20°C for the estimation of total protein. On day 45 post-insemination, all the cows were subjected to pregnancy diagnosis by palpation per rectum. Conception rate was calculated as percentage of animals that conceived following fixed time insemination in each group. The results on estrus response and conception rate following treatment was analysed by using Chi-square test while the values regarding biochemical parameters were analysed statistically through analysis of variance (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

All the animals had shown estrous signs following estrus induction hormonal treatment resulting in the estrus response of 100 percent. The result of the present trial concurs with the findings of Bhoraniya *et al*. (2012) and Dhami *et al*. (2015) who reported 100% estrus response in anestrus dairy cattle subsequent to estrus induction programme. However, in another study, 90 % estrus response was reported in crossbred anestrus cattle following estrus induction programme (Murugavel *et al*., 2010a). This difference may be due to the different combination of hormonal protocol with CIDR in the study, condition of the cow, efficacy of estrus detection, and other management practises adopted.

The mean time to onset of estrus from the time of removal of CIDR was 44.85 ± 7.23 h. The result in the present study agrees with the findings of Murugavel *et al.* (2010a) in anestrus dairy cows. The percentage of animals with weak, intermediate and intense estrum were 3.33 (1/30), 80 (24/30) and 16.67 (5/30) respectively. Majority of the animals (80%) have shown intermediate intensity of estrum following estrus induction. However, most of the previous studies (Sathiamoorthy and Kathirchelvan, 2010; Murugavel *et al*., 2010b) have shown that majority of the cows have exhibited intense estrus following CIDR treatment. Low percentage of cows showing intense estrus when compared to the previous studies could be due to low concentration of estrogen hormone in plasma to express estrus behaviour (Devipriya *et al*., 2020), difference in body condition and nutritional status of the animal (Butler, 2003).

The conception rate in the present study was 30 % (9/30). Previous studies have showed higher conception rates following estrus induction with CIDR ranging from 40.9% (Arab *et al.*, 2013) to 66% (Khade *et al*., 2011). In the present study, majority of the dairy cows showed intermediate intensity of estrus (80%) which might have associated with reduced pre-ovulatory estradiol concentration, delayed ovulation and poor oocyte quality compromising pregnancy rate (Cutullic *et al*., 2009). In the present study, the mean values of serum glucose, total protein and haemoglobin during estrus were 43.81 ± 1.14 mg/dl, 7.78 ± 0.24 g/dl and 8.68 ± 0.22 g/dl respectively. The mean glucose, total protein and haemoglobin levels in the present study are lower than the normal range in cows. The findings in the present study clearly indicate that the animals in the experimental trial were in poor nutritional status which may be the cause for the anestrus condition of the animal. Heuer (2000) reported that poor nutritional status is a major factor responsible for anestrus condition for the postpartum cows as the negative energy balance acts at various levels within the hypothalamus-pituitary-ovarian axis and which regulate follicular development and ovulation in animals (Armstrong *et al*., 2003).

The mean values of glucose, total protein and haemoglobin in cows that exhibited weak, intermediate and intense intensity of estrus are presented in Table 1. All the biochemical parameters among the different intensity of estrus were not statistically significant (P > 0.05). The glucose levels were lower in weak estrus animals when compared to the intermediate and intense estrus animals in the present study. The results are supported by Schneider (2004), who reported that hypoglycemia cause loss of ovarian activity by affecting hypothalamus and the release of gonadotropins from hypophysis.

Total protein levels were increasing with the increase in the intensity of the estrum in the present study. Vohra *et al*. (1995) observed that low level of plasma protein results in the deficiency of certain amino acids required for the biosynthesis of gonadotropins and gonadal hormones, which plays a major role in the expression of estrum.

The haemoglobin levels were increasing non-significantly with increase in the intensity of estrum in the present study. As the haemoglobin level increases, the animal will be in good health which might have improved the ability of the animal to exhibit the estrus signs.

The mean values of glucose, total protein and haemoglobin at the time of estrus for conceived (pregnant) animals and non-conceived (non-pregnant) animals are presented in Table 2. All the biochemical parameters between the conceived (pregnant) animals and non-conceived (non-pregnant) animals were not statistically significant (P > 0.05). There is slight difference in the levels of glucose between the pregnant (44.4±0.84 mg/dl) and non-pregnant (43.8±1.41 mg/dl) animals. Similar trend in a more pronounced manner is reported by Ghuman *et al*. (2011), who showed higher glucose level in the conceived animals when compared to non-conceived animals. The high blood glucose level increases the progesterone production directly by increasing LH pulse and mean concentration of LH and indirectly by increasing the blood insulin level, which stimulated progesterone secretion from luteal cells (Richards *et al*., 1989), which might have resulted in better the conception rate.

There is slightly high level of serum total protein in the pregnant (7.89±0.18 g/dl) when compared to non-pregnant (7.71±0.22 g/dl) animals. Ghuman *et al*. (2011) also found similar results i.e., total protein level for conceived and non-conceived animals. Kaitenbach and Dunn (1980) suggested that progesterone played a regulatory role in syn-

Table 1: Comparison of different parameters with intensity of estrus in Jersey CB cows.

Parameters	Intensity of estrum					
	Weak		Intermediate		Intense	
	n	Mean ± SE	n	Mean ± SE	n	Mean ± SE
Glucose (mg/dl)	1	35.00 ± 0.00	25	44.4 ± 1.14	4	44.40 ± 1.03
Total Protein (g/dl)	1	6.50 ± 0.00	25	7.75 ± 0.19	4	8.12 ± 0.22
Haemoglobin (g/dl)	1	8.00 ± 0.00	25	8.67 ± 0.19	4	8.92 ± 0.08

Table 2: Comparison of different parameters between pregnant and non-pregnant animals.

Parameters	Pregnant	Non-Pregnant	P - values	Significance
	Mean ± SE	Mean ± SE		
Glucose (mg/dl)	44.8 ± 1.1.9	44.4 ± 1.66	0.772	NS
Total Protein (g/dl)	7.99 ± 0.23	7.55 ± 0.22	0.624	NS
Haemoglobin (g/ml)	8.66 ± 0.22	8.72 ± 0.22	0.245	NS

thesis of specific amino acids. This might be the reason for increased demand for protein and amino acids for GnRH and LH synthesis during early luteal phase of the cycle in turn improving the conception rate.

Though statistically non-significant, there is a tendency of high level of haemoglobin in the pregnant (8.97±0.31 g/dl) when compared to non-pregnant (8.57±0.17 g/dl) animals in the present study. The results are in agreement with previous works who reported that level of haemoglobin was higher in pregnant animals that non-pregnant animals (Mirzadeh *et al*., 2010; Kumar and Sharma, 1993). As the haemoglobin is an indicator of health of the animals, good health tends to have higher conception rate. During estrus, sufficient concentration of haemoglobin in blood is required for the proper transportation of oxygen and nutrients to the vital organs including ovary (Hafez, 2000).

Further, the conception rate in cows which exhibited weak, intermediate and intense estrus was 0%, 25 % and 60% respectively. The conception rate of animals with weak, intermediate and intense estrum was 0.0 (0/1). The result of the present study shows there is a tendency of increase in the conception rate with the increase in the intensity of estrum. These differences are statistically non-significant as most of the animals are in the intermediate estrous group. Madureira *et al*. (2019), in his work reported that a strong estrous signalling was reflected in high pregnancy and calving rates. Similarly, Tippenhauer *et al*. (2021) stated that optimum expression of estrus is essential for better conception rate, which is directly proportional to the endocrine constitution of the animal at that stage. Lower intensity estrus has been associated with reduced pre-ovulatory estradiol concentration, delayed ovulation and poorer oocyte quality (Cutullic *et al*., 2009). Accordingly, a greater number of animals exhibiting intermediate estrus in the present study might be due to poor condition of the animals in turn affecting the hormonal state of the animal at the time of estrum.

CONCLUSIONS

From the present study, it can be concluded that nutritional status plays a major impact in prolonging the postpartum anestrus in crossbred cows. Biochemical parameters are positively associated with the intensity of induced estrus. Postpartum anestrus condition can be resolved by adopting progesterone-based protocol followed by timed AI.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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