International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2023; 8(4): 421-423

www.veterinarypaper.com
Received: 07-05-2023
Accepted: 12-06-2023

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Influence of body condition score and serum progesterone on conception rate following estrus induction in Anestrus cows

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Abstract
The present study was conducted to assess the influence of Body Condition Score (BCS) and serum progesterone concentration at the time of AI on intensity of estrus and conception rate in thirty anestrus crossbred cows following estrus induction. The cows were treated with progesterone intravaginal (CIDR) based estrus induction protocol with double fixed timed inseminations at 12 and 24 h after injection of last GnRH. BCS were recorded for the all the cows before estrus induction protocol. On the day of first AI, blood was collected from all the animals to estimate serum progesterone level. The overall conception rate in the present study was 30 % (9/30). The distribution of animals with BCS of 2.00, 2.25, 2.50, 2.75 and 3.00 were 6.67 %, 43.33%, 40%, 6.67% and 3.33% respectively. In the present study, there is trend that the intensity of estrus is increasing with increase in BCS. Though statistically non-significant, the conception rate was also found to increase with increase in BCS. The progesterone concentration at the time of induced estrus was found to be 0.59±0.00, 1.01±0.19 and 0.52±0.13 ng/ml in animals with weak, intermediate and intense estrum, respectively. The progesterone concentration at the time of estrus for pregnant cows (0.63±0.10 ng/ml) is significantly (p<0.05) lower when compared to non-pregnant cows (1.04±0.21 ng/ml). In conclusion, BCS positively associated with the intensity of induced estrus and serum progesterone level at the time of AI and pregnancy status is negatively correlated in anestrus cows following estrus induction.

Keywords: Postpartum anestrus, crossbred cows, estrus induction, BCS, progesterone concentration

Introduction
Anestrus in dairy cows is a functional disorder of the reproductive cycle which is characterized by complete sexual inactivity without manifestation of estrus [1]. In cattle following calving, there is a normal anestrus period. In *Bos indicus* cows, anestrus is considered as abnormal when it extends beyond an average of 90 days [2]. Following calving, there is deviation in follicular growth, selection of a dominant follicle, follicular maturation, ovulation, and followed by luteolysis, resulting in restoration of cyclical ovarian activity. The anestrus condition is associated with the presence of static ovaries, and even though there is follicular development, none of the ovarian follicles that start growing becomes mature enough to ovulate. As a result of this lack of follicular maturation, ovulation does not occur while anestrus is present [3]. Prolonged postpartum anestrus is one of the main infertility problems due to its great incidence, and results in economic losses to the dairy farmers due to failure to achieve a desirable inter-calving interval of 12 months [4]. 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 [5]. Assisted reproductive techniques used to hasten the re-establishment of cyclicity in the postpartum period may have a great impact on dairy farming. The commonly used treatments for the re-establishment of postpartum ovarian cyclicity, include use of progesterone or progestogens for short period in estrus induction programme. The progesterone releasing devices maintain plasma concentrations of progesterone for a given period of time. As progesterone concentrations reach sub-luteal levels during treatment, there is an increase in LH pulse-frequency, leading to follicular growth which, in turn, prevents atresia of the dominant follicle [6].

However, the responses to progesterone treatments are inconsistent and appear to be dependent on those factors influencing the prevalence of anestrus, such as age, body condition, endocrinological status and interval from calving [7]. Hence, the present research work was designed to the study the influence of body condition score and serum progesterone level at the time of induced estrus on conception rate following fixed timed AI in anestrus crossbred cows.

Materials and Methods

Thirty anestrus cross-bred cows reared by dairy farmers in the rural areas of Puducherry region were utilized for this study. All the animals were maintained under uniform management conditions, *viz*, grazing, substituted by stall feeding of fodder and concentrates. Cows have not shown signs of estrus even after 45 days postpartum and were without any palpable uterine disorders and not having CL on the ovaries on two successive per rectal examinations at 10 days interval. Body Condition Score (BSC) for all the animals was recorded at the onset of the treatment. The cows were scored for body condition on a five-point scale at 0.25 increments as per [8].

Cows were subjected to estrus induction using progesterone-based estrus induction protocol. CIDR (EAZI-BREED CIDR, Manufactured by DEC International Ltd; Hamilton, New Zealand and marketed by Pfizer Animal Health, Mumbai, containing 1.38g Progesterone) was inserted intravaginally on day 0 with or without GnRH (15 cows received GnRH on Day 0) and maintained for 7 days. Injection PGF2α (Pragma (Intas Pharmaceuticals Ltd; Ahmedabad, containing Cloprostenol @ 250 mcg/ml) 2 ml was administered intramuscularly on day 6 and injection GnRH (Ovulanta, Vet Mankind, New Delhi, containing Buserelin acetate @ 4 mcg/ml) 2.5 ml, was administered intramuscularly on day 9 to all the animals. The cows were inseminated with good quality frozen thawed semen at 12 and 24 h after the injection of last GnRH injection.

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 early morning, noon and early evening [9] to record the intensity of estrus and onset of estrus following treatment. Basing 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 [10]. 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 estrous signs. Blood samples were collected from jugular vein with the help of 18 gauge needle from all experimental animals on day 9 at the time of first AI into non-heparinized tubes and placed in ice box immediately. The non-heparinized blood was allowed to clot at room temperature, the serum was separated by centrifuging at 3000 rpm for 10 min and stored at -20 °C for the estimation of serum progesterone. Serum progesterone concentration was estimated using solid-phase Radio Immuno Assay technique with the help of progesterone kits (Coat-A-Count, Diagnostic products Corporation, USA). 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 at induced estrus in each group. The data was analysed statistically [11] using online SAS software version 20.00.

Results and Discussion

All the animals have shown the signs of estrus following estrus induction hormonal treatment resulting in the estrous response of 100 percent. 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. The distribution of animals with BCS of 2.00, 2.25, 2.50, 2.75 and 3.00 were 6.67 %, 43.33%, 40%, 6.67% and 3.33% respectively. The result shows that majority of cows are with BCS of 2.25 and 2.50. The comparison of BCS with intensity of estrus in presented in table 1.

Table 1: Comparison of Body Condition Score with Intensity of Estrum

Body Condition Score	Intensity of estrus							
	Weak		Intermediate		Intense		Overall	
	No. of cows	(%)	No. of cows	(%)	No. of cows	(%)	No. of cows	(%)
2	0	0	2	6.7	0	0	2	6.7
2.25	0	0	11	36.6	2	6.7	13	43.3
2.5	1	3.3	9	30	2	6.7	12	40
2.75	0	0	2	6.7	0	0	2	6.7
3	0	0	0	0	1	3.3	1	3.3

Chi-Square test: 7.418 P= 0.49
Non - Significance.

From the data, it can be envisaged that there is trend that the intensity of estrus is increasing with increase in BCS. However, the differences were statistically non-significant due to smaller experimental animals. The result of the present study agrees with the reports of [12, 13], who recorded that as BCS increased, signs of behavioral estrus were stronger and fertility has improved. Cows in a negative energy balance are reported to have lower levels of estrogen and progesterone and lower intensity of estrus [14].

The information regarding the correlation between BCS and conception rate is presented in table 2. 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% [15] to 66% [16]. The trial was conducted on cows maintained by the farmers with poor nutritional status and under field conditions.

Table 2: Comparison of Body Condition Score between pregnant and non-pregnant animals

Body Condition Score	Non-Pregnant	Pregnant	Conception Rate (%)
2	2	0	0
2.25	10	3	23.1
2.5	8	4	33.3
2.75	1	1	50
3	0	1	100
Total	21	9	30

Though statistically non-significant, the conception rate was also found to increase with increase in BCS. These results are in agreement with the findings of [17], who reported that the cows with less BCS had a lower pregnancy rate as compared to cows with high BCS. A 13 percentage unit increase in pregnancy rates for every unit increase in BCS was also reported previous work [12]. It has been shown that BCS or nutritional status of the female affects growth and follicular diameter [18], follicle maturation and ovulation [19] and in turn conception rate [20].

The progesterone concentration at the time of induced estrus

was found to be 0.59±0.00, 1.01±0.19 and 0.52±0.13 ng/ml in animals with weak, intermediate and intense estrum, respectively. These differences are statistically non-significant. Lower progesterone level and higher estrogen level shall improve the estrous intensity [21] but, in the present study most of the animals fell in to the intermediate intensity group, So the trend was not clear.

The progesterone concentration at the time of estrus for pregnant cows (0.63±0.10 ng/ml) is significantly (p<0.05) lower when compared to non-pregnant cows (1.04±0.21 ng/ml). The present result concurs with the findings of [22, 23] who reported lower level of progesterone concentration at the time AI in pregnant cows compared to nonpregnant cows. It was reported that higher level of progesterone at the time of estrus may be due to inadequate luteolysis prior to estrus resulting in anovulation [3] or might block the LH release and affect the oocyte maturation and ovulation and in turn affect the fertilization [24].

From the present study, it can be concluded that BCS positively associated with the intensity of induced estrus in anestrus crossbred dairy cows. Further, serum progesterone level at the time of AI and pregnancy status is negatively correlated in anestrus cows following estrus induction.

Acknowledgement

The authors are thankful to the Dean, Rajiv Gandhi Institute of veterinary Education and Research, Pondicherry for providing necessary facilities. The authors gratefully acknowledge the National Institute of Animal Nutrition and Physiology, Bangalore, India for the help in hormone estimation.

Conflict of interest

The authors declare no conflict of interest

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