Blood Plasma Progesterone Concentration In Postpartum Dairy Cows After Subjecting To Ovulation Synchronization With Triu-B® Progesterone Insert

Shuhaib, P.1; Leeba Chacko2*; Shynu, M.3; Amritha Aravind4

1M.V.Sc Student and 2,3,4Assistant Professor,
Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

*Correspondence: Leeba Chacko

ABSTRACT

The plasma progesterone concentration was estimated in postpartum anestrus dairy crossbred cows after subjecting to ovulation synchronization protocol with TRIU-B® progesterone insert with GnRH analogue and Estradiol benzoate. The mean serum progesterone level (ng/ml) on day 0 (pretreatment), at estrus, 10th day post estrus and 25th day post AI were recorded in all animals. The progesterone concentration was measured based on the principle of competitive ELISA. In EB group the mean serum progesterone level recorded on day 0 was 1.131 ± 0.488 ng/ml, on the day of estrus it was 0.484 ± 0.104 ng/ml, on day 10th post estrus it was 4.194 ± 0.350 ng/ml and on day 25th post AI it was 4.08 ± 0.702 ng/ml. In GnRH group, progesterone concentration on 0th day was 1.53 ± 0.3 ng/ml, at estrus 0.677 ± 0.047, on day 10 post estrus it was 3.636 ± 0.386 ng/ml and on day 25 post AI it was 2.84 ± 0.912 ng/ml. Along with progesterone assay pregnancy diagnosis was also carried out by using real time B-mode ultrasound scanner with multi frequency trans rectal probe (5.0–7.5 MHz) on 25th day post AI. The plasma progesterone concentration of 1ng/ml and above on test day indicated presence of luteal activity in ovary. The accuracy of diagnosis of non-pregnant animals at 25 day post AI by this method was 100 per cent.

Key words: postpartum anestrus, plasma progesterone, pregnancy

I. Introduction

The plasma progesterone assay could be used as a tool to differentiate true anestrus and sub-estrus animals (Honparkhe et al., 2008) and for monitoring the reproductive status in mammals (Muhammd et al., 2000). The usual methods of pregnancy detections such as visual detection, rectal palpation, service record and non-return to estrus are not fully reliable for diagnosing an early pregnancy. The concentration of progesterone in blood on 20 to 24 days post AI has been used as a tool for an early diagnosis of pregnancy in cattle (Ginther et al., 1976). Serum progesterone concentration 22 days post AI has been reported to range between 2.3 to 3.8 ng/ml in pregnant cows and between 0.1 to 2.6 ng/ml in non-pregnant Holstein Friesian multiparous cows with an accuracy of 83.3 per cent for pregnancy diagnosis on day 25 post AI (Muhammd et al., 2000). Also there are reports that in pregnant animals, the concentration of serum progesterone remain elevated on day 24, whereas in non-pregnant cows the serum progesterone concentration on day 24 decline to initial concentration (Alam and Ghosh 1994).

Therefore the present study was designed to assess the concentration of progesterone in peripheral blood of postpartum anestrus dairy cows on day 25 post AI after synchronizing estrus with
TRIU-B® progesterone insert (Device with 4 medicated rings, which supplies a total of 1 g of progesterone, Virbac Ltd.) and to correlate the result with that of trans-rectal ultrasound scanning done on the same day in an attempt to use it for early pregnancy diagnosis.

II. Materials and Methods

Postpartum anestrus dairy cows below eight years of age having body condition score of 2.5 to 3.5 and without any postpartum complication, in their first to fourth parity were selected from the Instructional Livestock Farm Complex, Pookode (ILFC) and Entrepreneurship and Veterinary Clinical Centre, Meenangadi. Animals were closely monitored for assessing their reproductive status through physical and clinico-gynaecological examinations. Postpartum animals starting from 40 days postpartum were screened by rectal examination and trans-rectal ultrasonography 12 days apart for analyzing ovarian functional status. Among these, 24 apparently healthy animals were selected and allotted randomly to two experimental groups and one control group comprising of eight animals each.

Experimental animals of group I were subjected to TRIU-B® + GnRH protocol followed by Fixed Time Artificial Insemination (FTAI) 56 hours after the removal of the insert. The group II animals were subjected to the TRIU-B® + estradiol protocol and FTAI was performed 48 h after removal of the insert. Animals which showed natural estrus after 40 days of calving were inseminated at the detected heat and treated as control.

Blood samples were collected from experimental and control animals prior to treatment (day 0), during induced or natural estrus, on 10th day of estrous cycle and on 25th day post AI for estimation of serum progesterone. The blood samples were kept overnight at 5°C and then centrifuged at 1500 rpm for 10 minutes for serum separation. The samples were stored in plastic vials at -20°C in a deep freezer until analysis.

The serum progesterone levels in the samples were analyzed using commercial ELISA kits (Pathozyme® Progesterone, Omega Diagnostics Ltd.). Pregnancy diagnosis was also carried out using real time B-mode ultrasound scanner with multi frequency trans-rectal probe (5.0–7.5 MHz) on 25th day post AI.

III. Results

The mean serum progesterone level (ng/ml) on day 0 (pre-treatment), at estrus, 10th day post estrus and 25th day post AI were recorded in all animals and shown in Table 1 and Fig. 1. Trans-rectal ultrasound scanning was performed along with blood collection on day 25 post AI to confirm pregnancy. All animals with high serum progesterone level (≥ 2 ng/ml) were confirmed pregnant by ultrasonography on day 25 post AI and rectal palpation on day 45 post AI.

The plasma progesterone levels of pregnant animals ranged between 3.33 to 4.99 ng/ml in TRIU-B® + GnRH protocol and 4.89 to 5.10 ng/ml in TRIU-B® + estradiol protocol on day 25 post AI in crossbred dairy cows after synchronization. In non-pregnant animals the level varied from 0.637 to 1.42 ng/ml in TRIU-B® + GnRH protocol and 0.637 to 1.29 ng/ml in TRIU-B® + estradiol protocol.

In the present study, all animals diagnosed pregnant by serum progesterone and trans-rectal ultrasonography at day 25 post AI were confirmed pregnant at day 45 by rectal palpation. Hence the accuracy was 100 per cent for non-pregnant animals and 87.50 per cent in pregnant animals.

| Table 1. Serum progesterone profile (mean ± SE) in postpartum crossbred dairy cows treated with different ovulation synchronization protocols |
|---------------------|---------------------|---------------------|---------------------|
| Groups             | Serum progesterone level (ng/ml) | Day 0 (ns) | At estrus** | Day 10 post estrus** | Day 25 post AI* |
| Group I            |                                   | 1.53 ±0.30 | 0.677 ± 0.04a | 3.636 ± 0.38a | 2.84 ± 0.91ab |
Group I: TRIU-B® + GnRH, Group II: TRIU-B® + Estradiol, Group III: Control

*Means having different letter as superscript within a column are significantly different (P≤0.05)
** Significant at 0.01 level; * Significant at 0.05 level; ns- Non significant

![Serum progesterone profile](image)

Fig. 1. Serum progesterone profile in postpartum crossbred dairy cows treated with TRIU-B® based ovulation synchronization protocols in comparison with control

IV. Discussion

Animals with plasma progesterone level ≥ 1ng/ml on the day of test (on day 25 post AI) were considered pregnant as described by Muhammed et al. (2000). In the present study, the plasma progesterone concentration was at basal level on the day of estrus and reached peak level on the day 10 of the estrous cycle in both pregnant and non-pregnant cows as described by Robinson et al. (2008), who reported that by day 4 post insemination circulating progesterone levels started to increase and it reached the maximum concentration by day 8 to 10. The result in the present study indicate that all animals in both the experimental groups responded to the synchronization protocols using with TRIU-B® progesterone insert with GnRH or estradiol benzoate.

Plasma progesterone concentration was at basal level on the day of estrus, by day 10 post estrus the serum progesterone concentration elevated significantly and continued at the elevated level till day 40 in conceived animals, where as normal cyclicity of progesterone was exhibited in non-conceived animals on day 20 post AI onwards (Ammu et al., 2012 and Mondal et al., 2006). In the present study cows treated with TRIU-B® + estradiol protocol were having higher progesterone concentration on day 10 post estrus and day 25 post AI than TRIU-B® + GnRH protocol. This may be due to improved estrus response, pre-ovulatory LH surge, ovulation and normal CL development and high conception rate in estradiol protocols (Pancarci et al., 2002). Mann et al. (1995) reported high incidence of early embryonic loss in dairy cows due to low concentration plasma progesterone Hence in animals treated with TRIU-B® + estradiol protocol, the elevated progesterone level in blood could be the reason for high conception rate.
Muhammad et al. (2000) reported 100 per cent accuracy for serum progesterone estimation in detection of non-pregnant animals and 83.3 per cent accuracy for diagnosing pregnant animals at day 25 post AI. In the present study, all animals diagnosed pregnant by serum progesterone and trans-rectal ultrasonography at day 25 post AI were confirmed pregnant at day 45 by rectal palpation. Hence the accuracy was 100 per cent for non-pregnant animals and 87.50 per cent in pregnant animals. The incorrect diagnosis of pregnancy on early days of sampling may be due to embryo loss as some 30 per cent of dairy cows lose their embryos by day 25 after mating (Lamming et al., 1989) or may be due to prolonged luteal activity. So in present study the higher accuracy due to absence of early embryonic lose in all conceived animals until day 45 post AI.

The present study authenticate that the blood progesterone assay combined with trans rectal ultrasound scanning on day 25 post AI give better results in early pregnancy diagnosing in estrus synchronized postpartum anestrus crossbred dairy cows. Also suggest that oestradiol based estrus synchronization protocol resulted in elevated progesterone level than GnRH based protocol in postpartum dairy cows.

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Bibliography