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# Application of Progesterone Profiles in the Assessment of Post Artificial Insemination Fertility in Cattle

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## *ABSTRACT*

The present study was conducted to investigate success of AI in relation to progesterone concentrations with respect to the timing of inseminations or heat detection in the field. Milk samples were collected for progesterone assay from 149 crossbred cows subjected to AI. The milk progesterone concentrations were estimated by RIA to access the reproductive status of the cows. On the day of AI, progesterone concentrations were less than 1.0 nmol/L (Low) in 51% samples and more than 3.00 nmol/L (High) in 18%. Rest 31 % samples showed intermediate progesterone (1.01 to 2.99 nmol/L) suggesting that these cows were not in proper heat at the time of AI. On the basis of two samples (day 0 and day 12 post AI), 50% cows had low progesterone concentrations on the day of AI and high in the second sample, indicating that these cows were inseminated during estrus and ovulated subsequently. Whereas, 12% cows had high progesterone in both the samples suggesting that at the time of AI these cows were either in the luteal phase or had luteal cyst. Remaining 37% cow had intermediate progesterone concentrations on both the occasions suggesting that they were having either developing or regressing or malfunctioning corpora lutea or partial luteal cyst. Perusal of observations on progesterone concentrations showed that 18% of inseminations were done during luteal phase and another 30% were done at some inappropriate time of heat, thus suggesting that high proportion (48%) of cows under field conditions were inseminated while not in proper heat.

***Key Words:*** Artificial Insemination, Computer application, Cattle, Fertility, Progesterone.

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# INTRODUCTION

Detection of estrus and proper timing of artificial insemination (Al) are two most important factors for achieving high fertility rates in dairy cattle. Unfortunately, the conception rate (CR) following Al under field conditions is much lower than expectations, which has direct economic implications on Indian dairy industry. Success of AI could be marred by a combination of several factors related to management and or nutrition. The precise etiology of increasing failure of AI, however is unknown. Achievement of acceptable fertility following Al requires identification of specific factors and application of appropriate interventions.

Monitoring success of AI through conventional methods viz., rectal palpation of genitalia and non-return rate has limited value. Milk progesterone evaluation is considered as the gold standard test for evaluation of the reproductive status in dairy cattle (Adriaens et al., 2017). However, the studies involving progesterone profile for assessing reproductive status under field condition are rare. The present study was, therefore, conducted to evaluate the application of progesterone estimation in monitoring the fertility outcome of inseminated cows.

# MATERIALS AND METHODS

The study was conducted using a mutually agreed common protocol through a network of 30 Veterinary Officers/inseminators at different planes of Punjab. A total of 149 crossbred cows presented for artificial insemination (AI) were examined clinically. Cows found in normal heat were inseminated using frozen semen. Non returning cows were examined per rectally for pregnancy diagnosis 60 days later.

From 149 cows under consideration, a total of three milk samples of 10 ml each at day 0 (at the time of AI), day 12 and day 21 were collected in the sterile glass vials containing 100 mg of sodium azide. These were centrifuged at 3000 rpm for 15 min and refrigerated for 10 min, so that the supernatant fat layer could get solidified. With the help of a syringe and a needle, the skimmed milk fraction was taken in 5 ml glass vials and stored at -20°C till further analysis. Progesterone was estimated using DPC (Diagnostic Product Corporation) coated RIA (Radio Immuno Assay) kits provided by IAEA (International Atomic Energy Agency).

Data on history, clinical observations and progesterone profiles were stored and analyzed using AIDA (Artificial Insemination Database Application), IAEA computer software designed to store and analyzed data related to AI.

# RESULTS AND DISCUSSION

## Monitoring success of AI through progesterone analysis

Milk progesterone was successfully analyzed in 447 samples collected on day 0, 12 and 21 post insemination. The cows were categorized as having either low (< 1.0 nmol/L), medium (1.1- 2.99 nmol/L) milk progesterone concentration on a particular day. Progesterone concentrations below 1 nmol/L have been considered to be either in heat, if associated with heat symptoms, or anestrus (Than et al., 1999). Dzung et al. (1999) reported progesterone concentration greater than 3.00 nmol/L in fat free milk of cows during luteal phase.

**Progesterone concentrations in milk sample on the day of insemination (day 0)**

On day 0, 76 (51%) cows had low milk progesterone concentration indicating a complete lack of luteal activity, 46 cows (31%) had high progesterone concentration and 27 (18%) were in the intermediate category of milk progesterone concentration. High progesterone cows were not in proper estrus at the time of AI and insemination in these cows was done during either luteal phase or in too early or too late in relation to estrus. Alternatively, these cows either had partially luteinized cysts or were in gestational estrus (Hafez, 1993).

**Progesterone concentrations in two milk samples taken on day 0 and day 12**

A total of 140 cows were milk sampled on day 0 and day 12. Of these, 70 cows (50%) had low progesterone concentration on day 0 and high in the second sample indicating that these cows had ovulatory estrus. Seventeen cows (12%) had high progesterone in both the samples suggesting the cows having either luteal phase/pregnancy or luteal cyst. Mongiardino et al., (1999) reported that 6% of inseminations were performed in cows with pregnancy, ovarian cysts or anestrus. The remaining 46 cows (37%) had intermediate progesterone concentration on the occasions suggesting that they were having a developing, regressing or malfunctioning CL or a partial luteal cyst during the course of sampling. Pedroso et al., (1999) noted that 33% of inseminations were performed on cows having anovulatory cycles, anestrus or short luteal phases. Toleng et al., (1999) observed low progesterone on day 0 and high on day 10-12 in 55.5% cow on large farm and in 58.6% in the

small farm. Anestrus, anovulation or short luteal phases were detected in 26% cow inseminated in large farms and 30.8% in small farms.

Progesterone concentrations in three samples (days 0, 12 and 21) and pregnancy diagnosis

A total of 103 cows were milk sampled thrice and were subjected to per rectal pregnancy diagnosis. The results are given in Table 1. Out of these, 40 cows showed low high-high progesterone combination with positive pregnancy diagnosis, indicating 39.6% overall pregnancy rate. Low-high-low progesterone combination with negative pregnancy diagnosis was found in 8 cows, indicating conception failure or early embryonic death in 7.7% cows. Cows losing their embryos between day 6 and 16 after breeding would normally return to estrus.

**Table 1.** Milk progesterone concentrations on Day 0, 12 and 21 post insemination and pregnancy status of 103 cows.

| Day 0 (AI) | Day 12 | Day 21 | Pregnancy diagnosis | Number (%) |
|---|---|---|---|---|
| Low | High | High | Positive | 40 (38.83%) |
| Low | High | Low | Negative | 08 (7.76%) |
| Low | High | High | Negative | 10 (9.71%) |
| High | High | High | Positive | 00 (0.00%) |
| Low | Low | Low | Negative | 00 (0.00%) |
| At least one sample had intermediate P4 conc. (1 to 2.99 nmol/L) | | | Positive | 09 (8.74%) |
| | | | Negative | 36 (34.95%) |

Low: < than 1 nmol/L, High: > than 3.0 nmol/L

Negative pregnancy diagnosis with low-high-high progesterone combination was present in 10 cows (9.7%) suggesting late embryonic mortality, luteal cysts or persistent CL, or prolonged cycles. Lamming and Darwosh (1998) reported 8% cows had estrous cycle with prolonged luteal phases.

Numerous factors affect the success of AI in dairy cattle and buffaloes. Singh et al. (2008) observed that quality of estrus, semen, management and expertise of AI technician have a strong bearing on the success of AI in cross bred cows. With respect to progesterone, Yan et al. (2018) stated that adequate but not excessive progesterone levels on day 5 bring about a better fertility, and plasma leptin concentration may be a much better indicator of metabolic status in lactating dairy cows. The life span of ovulatory follicle (Singh et al., 2009) had a strong bearing on outcome of AI. Madureira et al. (2021) observed that the high concentrations of P4 prior and low of concentrations of P4 at AI were associated with greater estrus intensity and pregnancy per AI at both spontaneous and timed AI events. Subclinical endometritis is also one of the factors affecting outcome of AI. Parenteral and intrauterine infusions of different antimicrobial agents, and immunomodulators and proteolytic enzymes (Singh et al., 2020) had met with variable success rate.

In this study, 45 cows (43.7%) had at least one sample with intermediate progesterone value. Out of these, 9 got pregnant and 35 were non pregnant. Pregnancy in theses 9 cows could be due to double AI practices at 12-24 hours interval adopted by some farmers. If 1st insemination was too early in these cows, the 2nd AI on next day might have compensated for that. For other 35 cows luteal deficiency or improper AI could be the suspected cause of conception failure.

## CONCLUSIONS

Perusal of data shows that untimely AI (up to 48%), as revealed by progesterone on day 0 probably due to inefficient estrus detection, remained as the major cause of repeat breeding in otherwise normal cycling cows. Detection of the estrus is still a major problem and error in detection of estrus account for large proportion of repeat breeding problem in dairy cows. The present study showed that progesterone analysis could provide vital information on fertility status and the progesterone on day 0 have high diagnostic value for detecting estrus.

## CONFLICT OF INTEREST

Authors don't have any conflict of interest.

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## REFERENCES

Adriaens, I., Huybrechts, T., Geerinckx, K., Daems, D., Lammertyn, J., Ketelaere, B. and Aernouts, B. (2017). Mathematical characterization of the milk progesterone profile as a leg up to individualized monitoring of reproduction status in dairy cows. Theriogenology, 103: 44-51.

Dzung, C. A., X, C. L., Ngoc, L. V., Van, C. D., Chung, D. P. and Hai, P. H. (1999). Constraints on efficiency of artificial insemination and effect of nutrition on reproductive performance of dairy cattle in small holder farms in Viet Nam. Proceedings of a Final Research Co-ordination Meeting organized by the Joint FAO/IAEA Division of Nuclear

Techniques in Food and Agriculture held in Uppsala, Sweden, pp. 67.

Hafez, E. S. E. (1993). "Reproductive behaviour", Reproduction in farm animals. 6th Edn Lea and Febiger, Philadelphia, 237-257.

Lamming, G. E. and Darwosh, A. O. (1998). The use of milk progesterone profile to characterise components of sub fertility in milked dairy cows. Animal Reprod. Sci., 52: 175-190.

Madureira, A.M.L., Burnett, T.A., Borchardt, S., Heuwieser, W., Baes, C.F. and Vasconcelos, J. L. M. (2021). Plasma concentrations of progesterone in the preceding estrous cycle are associated with the intensity of estrus and fertility of Holstein cows. Plos One, https://doi.org/ 10.1371/journal.pone.0248453.

Mongiardino, M. E., Rodriguez, L. and Vinals, D. (1999). Studies on the causes of inefficiency in artificial insemination systems in dairy cattle in Argentina. Proceedings of a Final Research Co-ordination Meeting organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture held in Uppsala, Sweden. pp. 79.

Pedroso, R., Roller, F., Gonzalez, N., Flipe, N. and Bravo, M. (1999). Factors that affect the quality and efficiency of artificial insemination in oestrous synchronization programmes in dairy cattle. Proceedings of a Final Research Co-ordination Meeting organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture held in Uppsala, Sweden, pp. 111.

Singh, H., Brar, P.S., Singh, N., Jan, M. H., Honparkhe, M. and Dhindsa, S.S. (2020). Effects of intra-uterine infusion of proteolytic enzymes on selected cytokine concentrations, uterine inflammation, and fertility in postpartum water buffalo with subclinical endometritis. https://doi.org/10.1016/j.anireprosci.2020.106335.

Singh, J., Ghuman, S.P.S., Honparkhe, M. and Singh, N. (2009). Investigations of dominant follicle development, estrus response, ovulation time and fertility in PRID- Treated anestrous buffalo heifers Indian J. Anim. Sci.,79(8): 773-777.

Singh, N., Nanda, A. S. and Jagir, S. (2008). Assessment of factors affecting conception rate in crossbred cows following artificial insemination under field conditions. Indian J. Anim. Rep., 29(2): 211-215.

Than, U., Aung, M. U., Su, S. K. D. and Y, H. W. U. (1999). Improvement of cattle production in Myanmar through the use of progesterone RIA to increase efficiency and quality of artificial insemination services. Proceeding of a Final Research Co-ordination Meeting organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture held in Uppsala, Sweden, pp. 45.

Toleng, L., Sonjaya, H., Yusif, M. and Hamid, A. (1999). The use of progesterone RIA to increase efficiency and quality of artificial insemination services of beef cattle in South Sulawesi, Indonesia. Proceeding of a Final Research Co-ordination Meeting organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture held in Uppsala, Sweden, pp. 37.

Yan, L. Y., Robinson, R. S., Shi, Z. D. and Mann, G. E. (2018). Milk progesterone on day 5 following insemination in the dairy cow: associated metabolic variables and reproductive consequences. S. Afr. J. Anim. Sci., 48:2 361-368.