# PERIPHERAL PLASMA PROGESTERONE CONCENTRATION DURING EARLY PREGNANCY IN HOLSTEIN FRIESIAN COWS

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#### ABSTRACT

The plasma progesterone concentration was measured in 18 normal cyclic Holstein Friesian cows following artificial insemination for diagnosis of early pregnancy. The blood samples were collected from 10 multiparous and 8 heifer cows on every 4th day following insemination until day 41 post insemination. The plasma progesterone concentration was measured with the help of direct enzyme immunoassay. The values of plasma progesterone concentration ranged between 2.3 to 4.0 ng/ml and 0.1 to 2.2 ng/ml in pregnant and non-pregnant groups, respectively. The plasma progesterone concentration of 1.0ng/ml and above on test days were taken as pregnant for both multiparous and heifer cows. The accuracy of this test for diagnosing early pregnancy was 71.4% on day 2 post insemination and 83.3% on day 25 post insemination. The non pregnancy diagnosis by this test was 100% accurate on all days of test. Subclinical endometritis and/or early embryonic deaths seen to interfere with exact diagnosis of pregnancy.

Keywords: Holstein Friesian cows, plasma progesterone, pregnancy

#### INTRODUCTION

The plasma progesterone assay is one of the most useful hormonal analysis for monitoring the reproductive status in mammals. The traditional methods of pregnancy diagnosis such as visual detection, rectal palpation, service record and non-return to estrous are not reliable sources of diagnosing an early pregnancy. A new method of pregnancy diagnosis in cattle/buffalo is getting popularity based on the level of progesterone in plasma or milk. The concentration of progesterone in blood and milk at 20-24 days post insemination has been used as a tool for an early diagnosis of pregnancy in cattle (Heap et al., 1973; Ginther et al., 1976) and in buffaloes (Arora et al., 1979).

Enough information was available on the levels of progesterone in the peripheral blood of cows through-out the estrous cycle (Henricks et al., 1970; Chenantt et al., 1975) with various frequencies of blood sampling. Therefore, the present study was planned to access the levels of progesterone in peripheral blood of Holstein Friesian multiparous and heifer cows during days 22 to 41 post insemination in order to standardize a protocol for early pregnancy diagnosis.

## MATERIALS AND METHODS

. A total of 18 animals were selected for this study maintained at the Livestock Experiment Station, Bhunikey (Pattoki), District Kasur. Animals were divided into two groups.

Group A = 10 normal cyclic Holstein Friesian multiparous cows

Group B = 8 nomral cyclic Holstein Friesian heifer

The blood samples (10 ml) were collected by jugular vein puncture from each animal in heparinized test tubes at day 22 and then at day 25 post insemination. These animals were closely monitored for returning into estrous. Those animals which showed heat symptoms were excluded from the study and those which did not show heat symptoms were continued for blood collection on every 4th day following insemination until day 41st post insemination.

Immediately after collection, the blood samples were centrifuged at 3000 rpm for 10 minutes and plasma was transferred into the screw capped test tubes. The plasma thus obtained was stored at -20°C until progesterone assay.

The plasma progesterone concentrations were estimated by direct Enzyme Immuno-Assay with the help of ultra progesterone ELISA kit Neogen Corporation, using ELISA microplate reader (Bio-Rad, USA).

## RESULTS AND DISCUSSION

The average ± SD values of plasma progesterone

concentrations in pregnant and non-pregnant Holstein Friesian multiparous cows are shown in Fig. 1. The plasma progesterone levels ranged between 2.3 to 3.8 ng/ml and between 0.1 to 2.6 ng/ml in pregnant and nonpregnant Holstein Friesian multiparous respectively. Similarly the values of the plasma progesterone concentrations in pregnant and nonpregnant Holstein Friesian heifer cows are shown in Fig. 2. The plasma progesterone levels ranged between 2.3 to 4.0 ng/ml and between 0.1 to 2.2 ng/ml in pregnant and non-pregnant heifer cows, respectively. The minimum level set for diagnosing pregnancy was 1.0 ng/ml as described by Adeyemo (1989). The progesterone concentrations remain higher than 1.0 ng/ml through out the sampling period in pregnant multiparous and heifer cows. While in non-pregnant multiparous and heifer cows, the progesterone levels were higher than 1.0 ng/ml on initial days of sampling but later on fell down than 1.0 ng/ml indicating early embryonic loss.

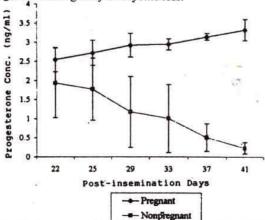


Fig. 1: Average ± SD values of plasma progesterone profile in pregnant and non-pregnant Holstein Friesian multiparous cows.

The accuracy of the progesterone test for pregnancy diagnosis based on the rectal finding is given in Table 1. The non pregnancy diagnosis by this test on all the test days was 100% accurate both in multiparous and heifer cows. Two multiparous and one heifer cows on day 22-25 and one multiparous cow on day 29-37 were wrongly diagnosed as pregnant, bringing the accuracy of the test for detecting pregnancy to 71.4% and 83.3%, respectively. The positive diagnosis of pregnancy on day 37-41 by the laboratory method agreed (100%) with the diagnosis by manual palpation of the uterus at that time. Similar findings have been found by Adeyemo (1989) in cows. The incorrect diagnosis of pregnancy on early days of sampling may be due to embryo loss as some 30% of dairy cows can lose their embryos by day 25 after mating (Lamming et al., 1989).

It is clear that the high incidence of early embryo loss in dairy cows is associated with low plasma concentrations of progesterone (Mann et al., 1995). Progesterone induces changes in the uterine environment conducive to conceptus growth and development (Geisert et al., 1992) and it is likely that if an embryo does not receive sufficient progesterone mediated stimulus, its development may be impaired.

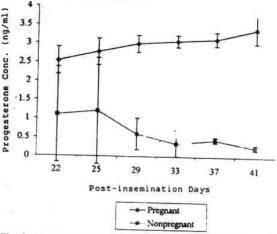


Fig. 2: Average ±SD values of plasma progesterone profile in pregnant and non-pregnant Holstein Friesian heifer cows.

The present findings suggest that taking blood samples after 25th day (i.e. 29th day) may give better results in Holstein Friesian cows irrespective of the parity status, since it covers in cows with long estrous cycles as well loss. These results extend over the results of Arora et al. (1979) and Kamonpatana et al. (1979) who used days 24 and 27 post-insemination respectively, for diagnosis of pregnancy in buffaloes.

Table 1: The accuracy of progesterone assays test for diagnosis of pregnancy in Holstein Friesian cows

COWS		
Test day	Laboratory Diagnosis	Accuracy (%)
Multiparous cows		
22-25 p.i.	Non Pregnant = 1	100
	Pregnant = 7	71.4
29-33 p.i	Non Pregnant = 2	100
	Pregnant = 6	83.3
37-41 p.i.	Non Pregnant = 3	100
	Pregnant = 5	100
Heifers cows		
22-25 p.i.	Non pregnant = 1	100
	Pregnant = 6	83.3
29-41-p.i.	Non pregnant = 2	100
	Pregnant = 5	100

### REFERENCES

- Adeyemo, O., 1989. Application of plasma and milk progesterone assay in pregnancy diagnosis in while fulami (Zebu) cattle. Anim. Reprod. Sci., 19: 205-208.
- Arora, R.C., N.K. Bachlaus., A. Prasad and R.S. Pandey, 1979. Plasma rogesterone levels in early pregnancy diagnosis in buffalo heifers. Ind. J. Exp. Biol., 17: 124.
- Chenantt, J.R., W.W. Thatcher, R.S. Kalra., R.M. Abrams and C.J. Wilcox, 1975. Transitory changes in plasma progestins, estradiol and leutinizing hormone approaching ovulation in the bovine. J. Dairy Sci., 58: 704-717.
- Geisert, R.D., G.L. Morgan, E.C. Short and M.T. Zary. 1992. Endocrine events associated with endometrial function and conceptus development in cattle. Reprod. Fertil. Dev., 4: 301-305.
- Ginther, O.J., I.C. Nuti, M.G. Garcia, B.G. Wentworth and W.J. Tyler, 1976. Factors affecting progesterone concentrations in cows milk and dairy products. J. Anim. Sci., 42: 155.

- Heap, R.B., M. Gwyn, J.A. Laxing and D.E. Waltrexs, 1973. Pregnancy diagnosis in cows; changes in milk progesterone concentration measured during estrous cyclic and pregnancy by radio immuno assay. J. Agri. Res., 81: 151.
- Henricko, D.M., J.F. Kickey and G.D. Niswender, 1970. Serum luetinizing hormone and plasma progestrone levels during the estrous cycle and early pregnancy in cows. Biol. Reprod., 2: 346-351.
- Kamonpatana, M.D., F.M. Van de Wikl., W. Koops, D. Leananuruksa, C. Ngramsuriyaroy and S. Vasanakernkul, 1979. Diestrous control and early pregnancy diagnosis in the swamp buffalo. Comparison of enzyme immunoassay and radio immunoassay for plasma progesterone. Theriogenology, 11: 399-406.
- Lamming, G.E., A.O. Darwash and L.H. Back, 1989. Corpus luteum function in dairy cows and embryo mortality. J. Reprod. Fertil. Suppl., 37: 246-252.
- Mann, G.E., G.E. Lammin and M.D. Fray, 1995. Plasma estradiol and progesterone during early pregnancy in the cow and the effects of treatment with buserelin. Anim. Reprod. Sci., 37: 121-131.