

# Ultrasonic Assessment of Corpora Lutea and Plasma Progesterone Levels in Early Pregnant and Non Pregnant Cows

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**Abstract**—Corpus luteum cross sectional (by ultrasonography) and plasma progesterone (by DELFIA) were estimated in early pregnant and non pregnant cows on days 14<sup>th</sup> and 20<sup>th</sup> to 23<sup>rd</sup> post insemination. On day 14<sup>th</sup>, corpus luteum sectional area was 348.43 mm<sup>2</sup> in pregnant and 387.84mm<sup>2</sup> in non pregnant cows. Within days 20<sup>th</sup> to 23<sup>rd</sup>, corpus luteum sectional area ranged between 342.06 and 367.90 mm<sup>2</sup> in pregnant and between 193.85 and 270.69 mm<sup>2</sup> in non pregnant cows. Plasma progesterone level was 2.43 ng/ml in pregnant and 2.46 ng/ml in non pregnant cows on day 14<sup>th</sup>, while during days 20<sup>th</sup> to 23<sup>rd</sup> the level ranged between 2.47 and 2.84 ng/ml in pregnant and between 0.53 and 1.17 ng/ml in non pregnant cows. Results of both luteal tissue areas as well as plasma progesterone levels were highly significantly deferent ( $P < 0.01$ ) between pregnant and non pregnant cows during days 20<sup>th</sup> to 23<sup>rd</sup>, but there were no significant differences on day 14<sup>th</sup>. The correlation between CL cross sectional area and plasma progesterone level was 0.4 in pregnant cows and 0.99 in non pregnant cow. It is clear, from this study, that ultrasonic assessment of corpora lutea is a viable alternative to determine plasma progesterone levels for early pregnancy diagnosis in cows.

**Keywords**—Progesterone, ultrasonography, corpus luteum, pregnancy diagnosis, cow.

## I. INTRODUCTION

IMPROVEMENT of reproductive efficiency in cattle has a great benefit to increase productivity and profitability. One of used protocols to improve reproductive efficiency in cattle is by early identification of pregnant and non-pregnant cows and decreasing the interval between services. Cattle man usually would like to know about pregnancy status of his cows as soon as possible after mating or insemination. Several methods are available for checking pregnancy of cows. Reference [1] stated that for successful integration into a reproductive management system, an ideal early pregnancy test for dairy cattle would be sensitive, specific, inexpensive, simple to conduct under field conditions, and able to determine pregnancy status at time of test is performed [1]. Most direct and indirect methods for pregnancy diagnosis exhibit one or more of these attributes, but none currently available exhibit all of them. The traditional test is rectal palpation. More recent innovations include ultrasonography scans, and blood tests. Uses of ultrasonography become more

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popular than last decade, for its importance in clinical diagnosis, developing of portable devices that can be easy to use in veterinary fields. Such methods can play a key role in reproductive management to rapidly return open animals to the breeding program. Several researchers studied concentrations of progesterone in different animals for its importance and its role in estrous cycle, maturation of ovulatory follicle, conception and maintenance of gestation. Some researchers have demonstrated that progesterone concentrations and the cross-sectional area of the CL were reduced in animals that were induced to ovulate prematurely [2], [3]. Others have noted decreased progesterone concentrations with ovulation of smaller follicles [4]. Within this study, trials to find a mirror to  $p_4$  concentration was applied to be a guide in early pregnancy diagnosis of inseminated cows; and the aim was to examine the relation between CL cross-sectional area by means of transrectal ultrasonography and  $P_4$  concentration profile of pregnant and non pregnant cows at day 14<sup>th</sup> and from day 20<sup>th</sup> to 23<sup>rd</sup> post insemination.

## II. MATERIALS AND METHODS

Eighty two Japanese Black cows that were used in the present study have lapsed at least 40 days after their last parturition. The cows were fed according to Japanese Feeding Standard [5]. Estrus synchronization was applied using single intramuscular injection of 500  $\mu$ g Prostaglandin F<sub>2</sub> $\alpha$  (PGF) analogues (RESIPRON-C, Teikoku Zoki Co.Ltd, Japan) to cows having functional CL. These cows were inseminated artificially by AM-PM protocol on day's 4<sup>th</sup> to 6<sup>th</sup> post injection [6]. Ovaries of all cows were scanned on day 14<sup>th</sup> and from day 20<sup>th</sup> until day 23<sup>rd</sup> using real-time ultrasonography (Tringa linear, Esaote piemedical, Netherlands) equipped with a 5 MH linear transducer. Blood samples were collected from jugular veins in heparinized tubes, immediately centrifuged at 1,670 x g for 20 minutes and were stored at -20°C until hormonal analysis was performed. Dissociation-Enhanced Lanthanide Fluorescent Immunoassay (DELFLIA) was used to determine plasma progesterone concentrations ( $P_4$ ) using progesterone antisera. Cross-sectional areas (mm<sup>2</sup>) of Corpora Lutea (CL c-s area) were calculated using the following formula:

$$\text{CL c-s area (Elliptical area)} = \pi \times (\text{diameter } a/2) \times \text{diameter } b/2,$$

where (a) and (b) are the long and short diameters of CL, respectively.

Statistical analyses: Significant differences between treatment and control groups were determined by  $\chi^2$  test or student's t test. P-value of less than 0.05 was considered statistically significant.

### III. RESULTS

Through monitoring ovaries and specifically the ovulatory follicle by ultrasonographic images, it was found that all used cows ovulated on the following day of artificial insemination. Thirty nine out of 82 cows were confirmed as pregnant on day 30<sup>th</sup> post insemination, while the remaining 43 cows were non-pregnant. CL c-s area on day 14<sup>th</sup> was  $348.43 \pm 9.29 \text{ mm}^2$  in pregnant and  $387.84 \pm 16.68 \text{ mm}^2$  in non pregnant cows. On days 20<sup>th</sup> to 23<sup>rd</sup> the CL c-s area was ranging between  $342.06 \pm 10.59 \text{ mm}^2$  and  $367.90 \pm 10.00 \text{ mm}^2$  in pregnant cows and it was significantly lower ( $P < 0.01$ ) between  $193.85 \pm 14.69 \text{ mm}^2$  and  $270.69 \pm 15.86 \text{ mm}^2$  in non pregnant cows (Table I). Mean P<sub>4</sub> concentration was  $2.43 \pm 0.14 \text{ ng/ml}$  in pregnant cows on day 14<sup>th</sup> and  $2.46 \pm 0.21 \text{ ng/ml}$  in non pregnant cows. However, mean P<sub>4</sub> concentration on days 20<sup>th</sup> to 23<sup>rd</sup> in pregnant cows ranged between  $2.47 \pm 0.21$  and  $2.84 \pm 0.21 \text{ ng/ml}$ . It was significantly lower ( $P < 0.01$ ), in non pregnant cows and ranging between  $0.53 \pm 0.16$  and  $1.17 \pm 0.21 \text{ ng/ml}$ , (Table II). Correlation was 0.4 (correlation coefficient) between P<sub>4</sub> and CL c-s area in pregnant cows, since P<sub>4</sub> tend to increase in pregnant cows after day 14<sup>th</sup> with relatively constant CL c-s area. In non-pregnant cows, the mean P<sub>4</sub> concentrations during days 20<sup>th</sup> to 23<sup>rd</sup> were significantly higher than those on days 14<sup>th</sup> ( $P < 0.01$ ). Correlation coefficient between P<sub>4</sub> concentration and the CL c-s area was 0.99 in non-pregnant cows (Figs. 1 and 2).

TABLE I  
CROSS SECTIONAL AREAS OF CORPORA LUTEA IN EARLY PREGNANT AND NON PREGNANT JAPANESE BLACK COWS

Observation	Pregnant	Non pregnant
	Mean $\pm$ S.E (mm <sup>2</sup> )	Mean $\pm$ S.E (mm <sup>2</sup> )
Day 14 <sup>th</sup>	$348.43 \pm 9.29$	$387.84 \pm 16.68$ b
Day 20 <sup>th</sup>	$367.90 \pm 10.00$ a	$270.69 \pm 15.86$ b
Day 21 <sup>st</sup>	$353.06 \pm 12.69$ a	$228.88 \pm 12.83$ b
Day 22 <sup>nd</sup>	$345.33 \pm 8.37$ a	$208.14 \pm 12.14$ b
Day 23 <sup>rd</sup>	$342.06 \pm 10.59$ a	$193.85 \pm 14.69$ b

Letters a, and b differ significantly ( $P < 0.01$ )

TABLE II  
PROGESTERONE PROFILES IN EARLY PREGNANT AND NON PREGNANT JAPANESE BLACK COWS

Observation	Pregnant	Non pregnant
	Mean $\pm$ S.E (ng/ml)	Mean $\pm$ S.E (ng/ml)
Day 14 <sup>th</sup>	$2.43 \pm 0.14$ a	$2.46 \pm 0.21$ b
Day 20 <sup>th</sup>	$2.84 \pm 0.21$ a	$1.17 \pm 0.21$ b
Day 21 <sup>st</sup>	$2.47 \pm 0.21$ a	$0.93 \pm 0.16$ b
Day 22 <sup>nd</sup>	$2.64 \pm 0.24$ a	$0.66 \pm 0.22$ b
Day 23 <sup>rd</sup>	$2.71 \pm 0.20$ a	$0.53 \pm 0.16$ b

Letters a, and b differ significantly ( $P < 0.01$ )

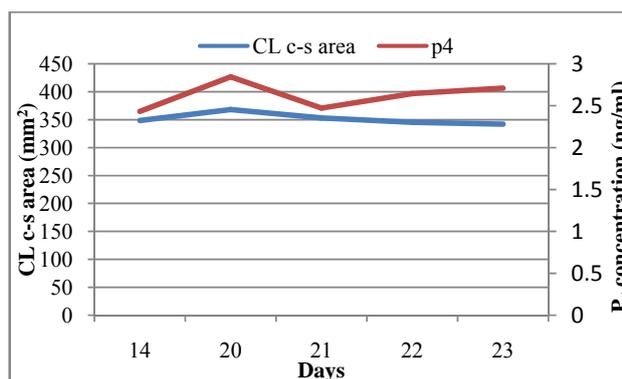


Fig. 1 Correlation between CL c-s area and P4 concentration in pregnant cows

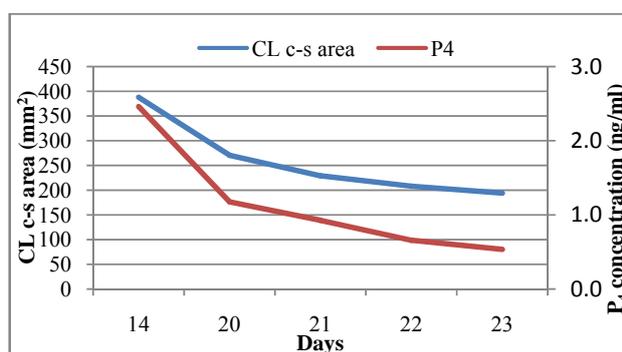


Fig. 2 Correlation between CL c-s area and P4 concentration in non pregnant cows

### IV. DISCUSSION

The current study was designed to correlate between CL cross-sectional area by means of Transrectal Ultrasonography and P<sub>4</sub> concentration profile of pregnant and non pregnant cows at day 14<sup>th</sup> and from day 20<sup>th</sup> to 23<sup>rd</sup> post insemination. This work is considered as a field application for our previous published research on early pregnancy diagnosis in Black Japanese cows [7]. The past mentioned work found that the cross sectional area of CL and plasma progesterone level during day 14, 15, 16 post insemination were the highest values in pregnant and non pregnant cows. Depending on that, the present work used day 14<sup>th</sup> of the cycle. It has been found that immediate results are possible, using Transrectal Ultrasonographic image of corpora Lutea cross-section areas (CLs C-S area), on days 13<sup>th</sup> & 20<sup>th</sup>, 14<sup>th</sup> & 21<sup>st</sup>, 15<sup>th</sup> & 22<sup>nd</sup>, and 16<sup>th</sup> & 23<sup>rd</sup> post insemination. Reference [8] reported results in a study which gave an early diagnosis of especially none conceived cow to be re-inseminated as early as possible without wasting more open-days [8]. Examination of plasma progesterone levels coupled with determination of CL cross-sectional area that are profiled over day 14<sup>th</sup> and from day 20<sup>th</sup> to 23<sup>rd</sup> post insemination provides an excellent overview of state of corpora lutea. So, the results which showed that CL c-s area on day 14<sup>th</sup> was  $348.43 \pm 9.29 \text{ mm}^2$  with P<sub>4</sub> level equal to  $2.43 \pm 0.14 \text{ ng/ml}$  in pregnant, and  $387.84 \pm 16.68 \text{ mm}^2$  with P<sub>4</sub> level equal to  $2.46 \pm 0.21 \text{ ng/ml}$  in non-pregnant cows;

which came almost similar in both pregnant and non pregnant cows and that is in agreement with those found by [7]. On days 20<sup>th</sup> to 23<sup>rd</sup> the CL c-s area was ranging between 342.06 ± 10.59 mm<sup>2</sup> and 367.90 ± 9.29 mm<sup>2</sup> with P<sub>4</sub> levels ranging between 2.47 ± 0.21 and 2.84 ± 0.21 ng/ml in pregnant cows and it was significantly lower (P<0.01) in non pregnant cows, which was ranging between 193.85 ± 14.69 mm<sup>2</sup> and 270.69 ± 15.86 mm<sup>2</sup> with P<sub>4</sub> levels ranging between 1.17 ± 0.21 and 0.53 ± 0.16 ng/ml. On the other hand, plasma progesterone levels were higher whenever CL c-s areas were larger; as it was clear in positively pregnant cows P<sub>4</sub> levels were always ≥ 2.2 ng/ml during days 20<sup>th</sup> to 23<sup>rd</sup> days of gestation, where as the levels were always < 1 ng/gm especially on days 21<sup>st</sup> to 23<sup>rd</sup> in non-pregnant cow. Such results is in agreement with results reported by [9] through their use of progesterone for early detection of open cows in dairy farms in Kenya [9]. However, with a series of samples taken at day 0 (the date of insemination), 21, and 24, the accuracy for early diagnosis of non-pregnancy approaches 95 to 100 [10], [11]. Therefore, progesterone test is a tool in the determination of the non-pregnant cow. The advantage of this early confirmation of non-pregnancy prevents the further loss of early breeding opportunities.

#### V. CONCLUSION

In conclusion, the use of this diagnostic tool increases the value of a veterinary clinic's services and enables more innovative veterinarians to maintain a progressive clientele. Ultimately, the use of ultrasound technology is financially advantageous for cattle producers and veterinarians in private practice; as it is clear from the results that ultrasonic assessment of corpora lutea is a viable alternative to determine plasma progesterone levels for early pregnancy diagnosis in cows.

#### REFERENCES

- [1] M. Fricke Paul, "Methods for Diagnosis and Monitoring of Pregnancy in Dairy Cattle and their Implementation," University of Wisconsin, Madison, WI, USA 2010.
- [2] C. R. Burke, M. L. Mussard, D. E. Grum, and M. L. Day, "Effects of maturity of the potential ovulatory follicle on induction of oestrus and ovulation in cattle with oestradiol benzoate," *Anim. Reprod. Sci.*, vol. 66, pp. 161–174, 2001.
- [3] J. L. Vasconcelos, R. Sartori, H. N. Oliveira, J. G. Guenther, and M. C. Wiltbank, "Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate," *Theriogenology*, vol. 56, pp. 307–314, 2001.
- [4] G. A. Perry, M. F. Smith, M. C. Lucy, J. A. Green, T. E. Parks, M. D. MacNeil, A. J. Roberts, and T. W. Geary, "Relationship between follicular size at insemination and pregnancy success," *Proc. Natl. Acad. Sci.*, vol. 102, pp. 5268–5273, 2005.
- [5] MAFF, Japanese Feeding Standard for Beef Cattle (2000). Edited by Agriculture, Forestry and Fisheries Research Council Secretariat. Japan Livestock Industry Association. Tokyo, 2004.
- [6] R. L. Nebel, W. L. Walker, M. L. Mc Gilliard, C. H. Allen, and G. S. Heckman, "Timing of artificial insemination of dairy cows: fixed time once daily versus morning and afternoon," *J. Dairy Sci.*, vol. 77, pp. 3185–3191, 1994.
- [7] A. Gaja, C. Kubota, and T. Kojima, "Early pregnancy diagnosis in Japanese black cows using a novel transrectal ultrasonography protocol," *Vet. Rec.*, vol. 164, pp. 659–660, 2009.
- [8] A. Gaja, S. Y. A. Al-Dahash, G. Raju, C. Kubota, T. Kojima, and I. Hatazoe, "Efficiency of Transrectal Ultrasonography Scanning of Corpora Lutea for Detecting Early Pregnancy in Cattle," (Poster). 27th World Buiatrics Congress, pp. 203–204, 2012.
- [9] D. Indetie, A. Indetie, J. Kinyua, N. Ojango, O. Perera, "Use of progesterone in early detection of open cows in smallholder dairy farms in Nakuru District of Kenya," In: Proceedings of the 4th All Africa Conference on Animal Agriculture. Dar es Salaam, Tanzania. 2006
- [10] R. L. Nebel, "On-farm progesterone tests," *J. Dairy Sci.*, vol. 71, pp. 1682–1690, 1998.
- [11] J. A. Pennington, L. H. Schultz, and W.F. Hoffman, "Comparison of pregnancy diagnosis by milk progesterone on day 21 and day 24 postbreeding: field study in dairy cattle," *J. Dairy Sci.*, vol. 68, pp. 2740–2745, 2005.