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Efficiency of measuring corpus luteum cross-sectional areas by ultrasonography for detecting early pregnancy in cattle

A.O. Gaja[1], S.Y.A. Al-Dahash[1], G. Raju[1], C. Kubota[2], T. Kojima[2], I. Hatazoe[3]

[1]Department of Surgery and Theriogenology, University of Tripoli, Tripoli, Libya.
[2]Laboratory of Theriogenology, Kagoshima University, Korimoto, Kagoshima, Japan.
[3]Kagoshima Agriculture Cooperative Associations, Kagoshima, Japan.

Abstract

The objective of this study was to examine the efficiency of a novel method of early pregnancy diagnosis in cows. This method was based on cross-sectional area changes of corpora lutea measured by transrectal ultrasonography. One hundred forty one Japanese Black cows were artificially inseminated and divided into four groups according to examination days. Corpora lutea of all cows in each group were measured twice at 7 days apart, on days 13 and 20 (n = 18), 14 and 21 (n = 40), 15 and 22 (n = 37) and 16 and 23 (n = 46) post-insemination. Confirmation of pregnancy was performed by ultrasonography on day 30 post-insemination. All non-pregnant cows showed clear regression of their corpora lutea at rates of 35.8, 43.7, 51.3, and 56.2% on days 20, 21, 22, and 23 post-insemination, respectively. Some ovarian disorders were discovered in 9% of non-pregnant cows. In conclusion, the present method was highly sensitive (100%) in detecting early non-pregnancies in the field, and also helpful in detecting ovarian disorders at an early stage, which gives reason to use it for better economic management and benefits.

Keywords: bovine, corpora lutea, early pregnancy diagnosis.

Introduction

The cattle industry depends mainly upon production of offspring and milk (De Vries *et al*., 2005). Early detection of non-pregnant cows after insemination is considered a valuable process in this industry in order to make a proper decision at the proper time. Several methods have been used for pregnancy diagnosis in cows, including the non-return method, rectal palpation, hormonal assay, transrectal ultrasonography, and other laboratory methods (Humblot *et al*., 1988; Cordoba *et al*., 2001; Katagiri *et al*., 2002; Isobe *et al*., 2005; Romano *et al*., 2006; Faustini *et al*., 2007; Mayer *et al*., 2013). Each method has its advantages and disadvantages. Cows that are diagnosed as non-pregnant after early pregnancy diagnosis at day 28 or 35 could be synchronized for estrus as soon as possible, or left waiting for the next natural estrus, which means wasting more time, money, and excessive efforts.

An early and accurate pregnancy diagnosis method that makes it possible to take suitable measures for the open cows may play a role in the success of the reproductive management of cattle. The most accurate, time saving, and cost beneficial method of pregnancy diagnosis is therefore important for optimum cattle farm management (Oltenacu *et al*., 1990; Thompson *et al*., 1995).

The aim of this experiment was to examine the efficiency of a novel, field applicable method of early pregnancy diagnosis in inseminated cows. This method was based on comparing the value of corpus luteum cross-sectional areas on their maximum growing days (days 13 to 16) with the value of corpus luteum cross-sectional areas around the vicinity of the days of prospective estrus (days 20 to 23) of the same estrous cycle by means of transrectal ultrasonography (Gaja *et al*., 2009).

Materials and Methods

One hundred forty one Japanese Black cows (within a cattle farm in Sendai City, Japan) were used for this work. Feeding at this farm was according to the standard Japanese Animal Feeding Programs (Agriculture Forestry and Fisheries Research Council Secretariat, 2004). All cows (n = 141) received intramuscular double injections of 500 µg Prostaglandin F2α analog (PGF2α; Resipron-C, Teikoku Zoki Co. Ltd., Japan) 11 days apart, after at least day 40 postpartum. Artificial insemination by a.m.-p.m. rule was performed on days 4-6 post-injection of the second dose of PGF2α, when the cow showed signs of estrus (Nebel *et al*., 1994).

For monitoring the growth and regression of corpora lutea (CL), ovaries of all cows were scanned three times during the experimental period using real-time ultrasonography (Tringa linear, Esaote Piemedical, Netherlands) equipped with a 5 MHz linear transducer. According to the times of scanning, the cows were divided into 4 groups, as follows:

Group 1: Cows scanned on days 13, 20, and 30 post-insemination; (n = 18).

Group 2: Cows scanned on days 14, 21, and 30 post-insemination; (n = 40)

Corresponding author: drgajja@yahoo.com
Phone: +21(891)491-3578
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Group 3: Cows scanned on days 15, 22, and 30 post-insemination; (n = 37).

Group 4: Cows scanned on days 16, 23, and 30 post-insemination; (n = 46).

The third scanning was conducted on day 30 post-insemination in all cows from all groups to confirm pregnancy. All ultrasonography scanning was performed gently for all cows used in this experiment by the same expert person, avoiding invasive manipulation of reproductive organs.

The cross-sectional areas (mm^2) of CL (CL c-s area) were calculated by the following formula: *CL c-s area (Elliptical area)* = $\pi \times$ *(diameter a/2)* $\times$ *(diameter b/2)*. Where a and b are the long diameter and the short diameter of the CL, respectively.

Regression rate of the CL was calculated by the following formula: *100 - (Day X_2 CL c-s area/Day X_1 CL c-s area)* $\times$ *100*. Where X_1 and X_2 represents the 1st and 2nd scanning days, respectively.

Significant analyses were determined by χ^2 test or student's t-test. A P-value of less than 0.05 was considered statistically significant.

Results

All cows from the four experimental groups (n = 141 cows) showed signs of estrus and were inseminated artificially, based on the am-pm rule. Based on the ultrasonographic examination on day 30 post-insemination, 62 cows (44%) were diagnosed as pregnant whereas the rest (79 cows; 56%) were empty. During the 1st ultrasonographic examination of the inseminated cows (days 13 to 16), some ovarian disorders were discovered in 13 cows (9%), which were all non-pregnant. These cows were excluded from the experiment and sent to treatment. The rest of non-pregnant cows (n = 66; 47%) showed significant regression in their corpora lutea at rates of 35.8, 43.7, 51.3, and 56.2% on days 20, 21, 22, and 23 post-insemination, respectively. Table 1 and Fig. 1 show the regression rates of corpora lutea in the non-pregnant cows at the 1st and 2nd ultrasonographic examinations. Pregnant cows had progressive increases in the development of their corpora lutea throughout the successive examination days (1st and 2nd ultrasonographic examinations). Table 2 shows the proportion of pregnant cows with CL cross-sectional area changes.

Table 1. Measurements of cross-sectional areas of corpora lutea in non-pregnant cows at 1st and 2nd ultrasonographic examinations.

Group	No.	Days	Mean (mm^2)	Std (mm^2)	Regression rate (%)
1	8	13	382.6	131.6	-
		20	234.4	73.4	35.8
2	19	14	367.9	92.9	-
		21	202.5	62.1	43.7
3	17	15	366.5	78.7	-
		22	175.4	52	51.3
4	22	16	350.5	94.5	-
		23	157.5	60.5	56.2
	66				

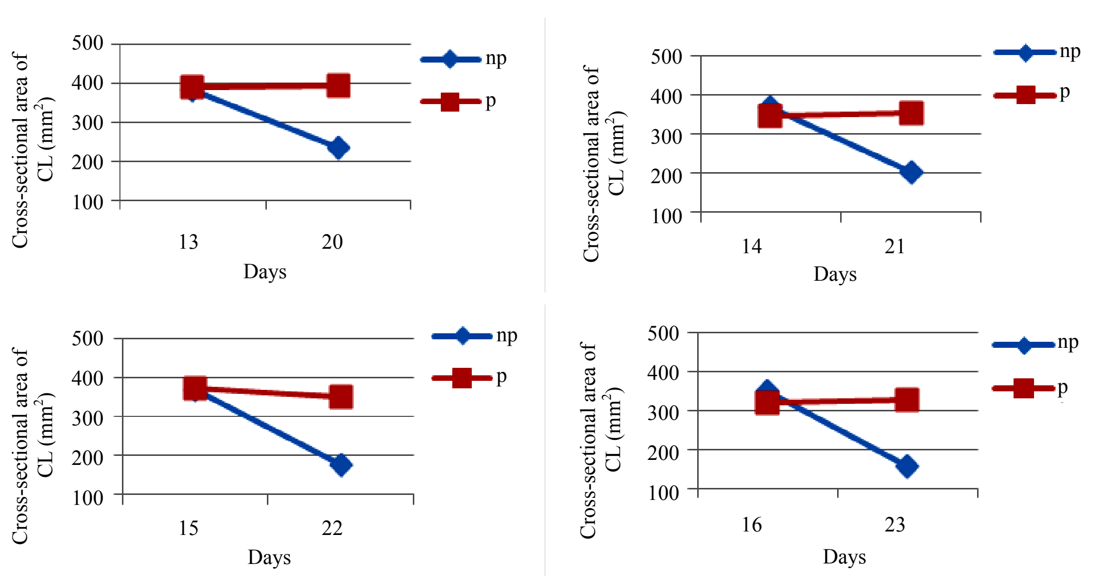

Figure 1. Cross-sectional areas of corpora lutea in pregnant and non-pregnant cows at 1st and 2nd ultrasonographic examinations.

Table 2. Proportion of pregnant cows with CL cross-sectional area changes.

Days of examination	Group	Total (No.)	CL area changes			
			Increased	Decreased No. (%)		
			No. (%)	up to 10%	10-25%	over 25%
13 and 20	1	7	4 (57.1%)	2 (28.6%)	1 (14.3%)	0 (%)
14 and 21	2	18	11 (61.1%)	5 (27.8%)	2 (11.1%)	0 (%)
15 and 22	3	16	5 (31.3%)	6 (37.5%)	5 (31.2%)	0 (%)
16 and 23	4	21	15 (71.4%)	3 (14.3%)	3 (14.3%)	0 (%)
	Total	62	35 (56.5%)	16 (25.8%)	11 (17.7%)	0 (%)

Discussion

This work is considered as a field application of our previous published research on early pregnancy diagnosis in Japanese Black cows (Gaja *et al*., 2009). Several studies have been done to evaluate economical profits through reproductive management (Oltenacu *et al*., 1990; Olynk and Wolf, 2008; Giordano *et al*., 2011). Increasing the number of open days of cows by any means negatively affects the economy of the herd (French *et al*., 2003, de Vries *et al*., 2004). On the other hand, non-pregnant cows that do not return to estrus have been termed "phantom cows" (Lucy *et al*., 2004). The presence of phantom cows creates a serious reproductive challenge. Under traditional reproductive management, a phantom cow is not detected until pregnancy examination within days 40 to 60 after initial insemination.

The present study aimed to reduce the number of open days through early pregnancy diagnosis. It has been found that immediate results are possible using transrectal ultrasonographic images of corpus luteum cross-section areas (CL c-s area) on days 13 and 20, 14 and 21, 15 and 22, and 16 and 23 post-insemination. The results of this study gave an early diagnosis of especially non-pregnant cows to be re-inseminated as early as possible without wasting more open days. The previous correlated work (Gaja *et al*., 2009) detected the relationship between regression rate of CL c-s area on days 14 and 20 and pregnancy status through ultrasonographic measurement of the CL c-s areas, considering that day 14 was the 100% highest value; so, rate of CL c-s area's change on day 20 may tell the possibility of positive or negative results for early pregnancy diagnosis. As suggested by Gaja *et al*. (2009), a practical technique that could be applied in the field to differentiate non-pregnant from pregnant cows, as early as possible without wasting time waiting for the next estrus should be proposed. We have shown that inseminated cows are definitely non-pregnant if the regression of their corpora lutea exceeded 25% within each of two ultrasonographic examinations. If the regression was less than 10%, the cow was considered as most likely pregnant. Gaja *et al*. (2009) reported no differences in CL c-s areas within days 13 to 16 post-insemination. Also, Taylor *et al*. (1991) reported no difference between pregnant and non-pregnant cows within similar periods. Therefore, it is a good option to choose any of the range periods; which means that the 1st ultrasonographic examination for CL c-s area could be applied any time within this period followed by a second examination within days 20 to 23 post-insemination. The result of the present study showed full agreement with the above work in application of the first ultrasonographic examination within days 13 to 16 post-insemination as the highest CL c-s area's value with days of 20 to 23 of the second ultrasonographic examination.

In conclusion, the present study, using transrectal ultrasonography to measure the changes in CL c-s area on days 13 to 16 post-insemination as a 1st exam and days 20 to 23 post-insemination as a 2nd exam, insured a high efficiency rate in detecting non-pregnant cows (100% in the second examination, during days 20 to 23 post-insemination), as well as detecting ovarian disorders at early stages. This approach can be used for better economic management and benefits.

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References

Agriculture Forestry and Fisheries Research Council Secretariat. 2004. *Japanese Feeding Standard for Dairy Cattle*. Tokyo: Japan Livestock Industry Association. 189 pp.

Cordoba MC, Sartori R, Fricke PM. 2001. Assessment of a commercially available early conception factor (ECF) test for determining pregnancy status of dairy cattle. *J Dairy Sci*, 84:1884-1889.

De Vries A, Van Leeuwen J, Thatcher WW. 2004. Economic importance of improved reproductive performance. *In*: Proceedings 2004 Florida Dairy Reproduction Road Show, Gainesville, FL. Gainesville, FL: UFL/IFAS Extension. pp. 33-42.

De Vries A, Bartolome J, Broaddus B. 2005. What is early pregnancy diagnosis worth? *In*: Proceedings of the 2nd Florida Dairy Road Show, 2005, Gainesville, FL. Gainesville, FL: UFL/IFAS Extension. pp. 31-40.

Faustini M, Battocchio M, Vigo D, Prandi A, Veronesi MC, Comin A, Cairoli F. 2007. Pregnancy diagnosis in dairy cows by whey progesterone analysis: an ROC approach. *Theriogenology*, 67:1386-1392.

French PD, Nebel RL. 2003. The simulated economic cost of extended calving intervals in dairy herds and comparison of reproductive management programs. *J Dairy Sci*, 86(suppl. 1):54. (abstract).

Gaja A, Kubota C, Kojima T. 2009. Early pregnancy diagnosis in Japanese black cows using a novel transrectal ultrasonography protocol. *Vet Rec*, 164:659-660.

Giordano JO, Fricke PM, Wiltbank MC, Cabrera VE. 2011. An economic decision-making support system for selection of reproductive management programs on dairy farms. *J Dairy Sci*, 94:6216-6232.

Humblot P, Camous S, Martal J, Charlery J, Jeanguyot N, Thibier M, Sasser G. 1988. Diagnosis of pregnancy by radioimmunoassay of a pregnancy-specific protein in the plasma of dairy cows. *Theriogenology*, 30:257-267.

Isobe N, Akita M, Nakao T, Yamashiro H, Kubota H. 2005. Pregnancy diagnosis based on the fecal progesterone concentration in beef and dairy heifers and beef cows. *Anim Reprod Sci*, 90:211-218.

Katagiri T, Sakai K, Sasaki K, Adachi Y, Tajima A, Honma H. 2002. Early pregnancy diagnosis using an enzyme-immuno assay kit for blood progesterone in dairy cattle. *Bull Agric Forestry Res Center*, 15:37-42.

Lucy MC, McDougall S, Nation DP. 2004. The use of hormonal treatments to improve the reproductive performance of lactating dairy cows in feedlot or pasture-based management system. *Anim Reprod Sci*, 82:495-512.

Mayer J, Soller JT, Beck J, Purwins V, Wemheuer W, Schutz E, Brening B. 2013. Early pregnancy diagnosis in dairy cows using circulating nucleic acids. *Theriogenology*, 79:173-179.

Nebel RL, Walker WL, McGilliard ML, Allen CH, Heckman GS. 1994. Timing of artificial insemination of dairy cows: fixed time once daily versus morning and afternoon. *J Dairy Sci*, 77:3185-3191.

Oltenacu PA, Ferguson JD, Lednor AJ. 1990. Economic evaluation of pregnancy diagnosis in dairy cattle: a decision analysis approach. *J Dairy Sci*, 73:2826-2831.

Olynk NJ, Wolf CA. 2008. Economic analysis of reproductive management strategies on US commercial dairy farms. *J Dairy Sci*, 91:4082-4091.

Romano JE, Thompson JA, Forrest DW, Westhusin ME, Tomaszweski MA, Kraemer DC. 2006. Early pregnancy diagnosis by transrectal ultrasonography in dairy cattle. *Theriogenology*, 66:1034-1041.

Taylor C, Rajamahendran R. 1991. Follicular dynamics and corpus luteum growth and function in pregnant versus nonpregnant dairy cows. *J Dairy Sci*, 74:115-123.

Thompson JA, Marsh WE, Etherington WG, Momont HW, Kinsel ML. 1995. Evaluation of the benefits of the timing of pregnancy testing by transrectal palpation in dairy cattle. *J Am Vet Med Assoc*, 207:1462-1465.