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A COMPRATIVE STUDY ON PREGNANCY DIAGNOSING USING TRANSRECTAL ULTRASONOGRAPHY, PROGESTERONE LEVEL AND EARLY CONCEPTION FACTOR ASSAY IN DAIRY COWS

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ABSTRACT: ***This study aimed to compare using of the early pregnancy factor (ECF), the progesterone (P4) level and the trasnsrectal ultrasonography (US) tests in determination of early pregnancy diagnosis in dairy cattle. Thirty two Holstein cows aged 4-8 years were involved in this study. Blood samples were collected from each cow on days 7 and 14 for detecting ECF and on days 21 and 24 after breeding for P4 determination. Pregnancy was diagnosed by ECF test, assaying P4 in serum and US technique. The three methods of were evaluated based on the result of the rectal palpation on day 50 after breeding. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of each test were calculated.***

Results indicated that using ECF test on day 7 after breeding revealed that 2 cows of the 14 pregnant cows diagnosed pregnant by rectal palpation were non-pregnant and later on day 14 after breeding was able to identify all the 14 pregnant cows. Thus the sensitivity of the test was 85.7% on day 7, reached 100% on day 14 post breeding. The incidence of the false-negative results was 14.3% and zero% on days 7 and 14 post breeding, respectively.

Using the ECF test on days 7 and 14 after breeding determined 4 and 3 cows of the 18 non-pregnant cows diagnosed by rectal palpation were pregnant, respectively. The specificity of the test was 77.6% on day 7, increased to 83.3% on day 14 after breeding. The incidence of the false-positive results decreased from 22.2% on day 7 to 16.7% on day 14 post insemination.

Using progesterone test on days 21 or 24 after breeding was successfully able in detecting all the pregnant cows. The sensitivity of the P4 test was 100% in identifying the pregnant animals. In contrast, the P4 assay failed in detecting the non-pregnant cows with the same sensitivity. The P4 test specificity was lower on day 21 (55.6%) than that on day 24 (66.7%) post breeding. the incidence of the false-positive results was very high (44.4% and 33.3% on days 21 and 24 after breeding, respectively). The transrectal US was very effective method in detecting all the non-pregnant cows. While it determined one cow of the 14 cows diagnosed pregnant by palpation was non-pregnant. It could be concluded that using ECF test on day 14 after

breeding seems to be more effective than that on day 7 after breeding in diagnosing pregnancy in cattle with accuracy 81.3-90.6% and could be conducted as a valuable method in culling and rebreeding of non-pregnant cows early after A.I. besides being as a valuable tool for controlling breeding program.

Keywords: ***Pregnancy diagnosis, Ultrasonography, Early conception factor, Cattle.***

INTRODUCTION

An early and accurate pregnancy diagnosis may play a fundamental role in the control of infertility in dairy cows by early detection of conception failure and identification of non pregnant animals which facilitates treatment, rebreeding or culling and saving the costs of maintaining these animals (Fricke, 2002). Various methods have been used for diagnosing pregnancy in dairy cattle including the clinical methods such as rectal examination and transreactal ultrasonography techniques as well as the chemical methods such as progesterone assay and pregnancy associated glycoprotein. Most of these methods are capable of detection of pregnancy in dairy cows between 25 to 40 days after insemination. Rectal examination of the genital tract is probably the most widely used method for pregnancy diagnosis in dairy cows as early as 35 to 39 days, although much practice is necessary to be able to diagnose pregnancy at that stage.

Data available in the literature indicated that pregnancy can be accurately diagnosed using transerectal ultrasonography (US) techniques as early as 26 days post breeding (Filteau and DesCoteaux, 1998 and Kastelic *et al*. 1991). However, sensitivity and specificity of pregnancy diagnosis using US were 44.8% and 82.3%, respectively when it was conducted between 21 and 25 days after breeding. They increased to 97.7% and 87.7%, respectively, when it was conducted between 26 and 33 days post breeding (Pieterse *et al*. 1990). It is worthy mentioning that the cost of the equipment and the special training and experience required may limit its practical implementation in dairy farms (Fricke, 2002). Assessment of progesterone (P4) in milk and blood have been also used to determine pregnancy in dairy cattle between 18 to 24 days after breeding with specificity in detecting non-pregnant animals around 98% (Pennington *et al.* 1985 and Nebel *et al*. 1987). However, the sensitivity of detecting pregnant animals in these studies was markedly lower than specificity. This difference may be due to embryonic mortality. It has been concluded that high P4 concentration during this period is not a specific indicator of pregnancy due to individual variation among cows in estrous cycle duration and embryonic mortality (Thatcher *et al*. 2001 and Wolf *et al*. 2003).

The early pregnancy factor (EPF) was identified in pregnant sheep and cattle by using the rosette inhibition bioassay (Nancarrow *et al*.. 1981), appeared in the serum of the pregnant cattle within 24 to 48 hrs of fertilization and disappeared within 24 to 48 hrs after death or removal of the embryo (Morton *et al*. 1987). Cavanagh, (1996) reported that EPF is a secreted substance with growth regulatory and immunomodulatory properties that is required for successful establishment of pregnancy. The early conception factor (ECF) test reportedly detects a pregnancy-associated glycoprotein within 48 h of conception (Concepto Diagnostics, Knoxville, TN). Threlfall, (1994) reported that ECF test is a lateral technology for measurement of the presence of the ECF glycoprotein in serum collected from pregnant cows between 6 and 15 days after breeding, and may have potential for the reduction of calving interval by providing a very rapid method of pregnancy diagnosis. Adams and Jordon (1999) and Des Coteaux *et al*. (2000) reported a poor accuracy for this commercial test in cattle due to the high rate of obtained false–positive results.

The aim of this study is to compare the accuracy of using of transrectal ultrasonography, progesterone concentration and detection of ECF in blood serum for diagnosing pregnancy in dairy cattle.

MATERIALS AND METHODS

Thirty two postpartum Holstein cows with no histories of reproductive disorders were involved in this study. Cows were aged 4-8- years and housed in free-stall pens at the Dairy Facility Center, The Ohio State University, Columbus, Ohio, USA during the period from May to October. The animals were fed a total mixed diet consisting of alfalfa hay, corn silage, soybean meal, whole cotton seed, megalac and cargill to meet NRC (1987) recommendation. They were milked twice daily. Close observation was conducted twice daily as normally occurs at the facility in order to detect estrous manifestation. As a cow was detected in standing estrus, she was artificially inseminated after about 12 hours, the day of insemination was considered as day 0.

Three different methods namely; ECF, P4 level and transrectal US in comparing with the rectal palpation were tested. In this respect, blood samples were collected via jugular vein puncture from each cow on days 7, 14 for detection of ECF glycoprotein as well as on days 21, 24 post-insemination for determination of progesterone. Blood samples were stored at 4 °C for 24 hrs to allow optimal coagulation and centrifuged at 3000 rpm for 15 minutes. Serum was harvested and stored at -20°C until progesterone determination and assaying of ECF test.

Progesterone was analyzed in serum samples using a double antibody radioimmunoassay (Anderson and Day, 1994). The cow was considered positive (pregnant), when the concentration of progesterone of her sample was >1 ng/ml.

The test of ECF was performed by placing one drop of the serum using a supplied dropper pipette into the sample well followed by addition of 4 drops of running buffer. After 2 hours incubation at room temperature, the presence of tow lines indicates that the cow has been already conceived (Concepto Diagnostics Corporation, early conception factor dipstick test (ECF) for cattle, Knoxville TN).

The examination of the transrectal ultrasonography was performed on day 30 post insemination using an Aloka 500 ultrasound unit with a 7.5 MHz transducer. Cows were restrained in the standing position and the rectum was cleared of feces. The ultrasound probe was introduced into the rectum and moved over both uterine horns to find the embryo vesicle, the embryo itself and to detect its heartbeats or movements.

To confirm the status of pregnancy, each cow was rectally examined on day 50 after insemination. Based on the result of the rectal palpation, the three methods of the pregnancy diagnosis (ECF, P4 and US) were evaluated.

Data obtained were statistically analyzes by Chi-squares using SPSS (1997) program. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of each test were calculated as described by Cordoba *et al*., 2001 as follow:

$$\text{Test sensitivity} = 100 \times \frac{A}{A+B} \qquad \text{PPV} = 100 \times \frac{A}{A+D}$$

$$\text{Test specificity} = 100 \times \frac{C}{C+D} \qquad \text{NPV} = 100 \times \frac{C}{C+B}$$

$$\text{Test accuracy} = 100 \times \frac{A+C}{A+C+D+B}$$

A: True positive results (pregnant). C: True negative results (non-pregnant).

B: False negative results (non-pregnant) D: False positive results (pregnant).

RESULTS AND DISCUSSION

Examination through rectal palpation on day 50 after breeding revealed that 14 cows were pregnant and 18 cows were non-pregnant. Data in Table 1 indicated that using ECF test for diagnosing pregnancy in serum of dairy cows on day 7 after breeding have succeeded to detect only 12 cows being pregnant. However, using the same test later on day 14 after breeding was able to diagnose correctly all the 14 pregnant cows, which agrees with the results obtained by rectal palpation.. The pregnant animals may have been determined as non-pregnant (as occurred on day 7 post breeding, Table 1) could be due to a delay in fertilization or production of the ECF by the fertilized oocyte (Threlfall, 1994). The sensitivity of the ECF test which measures the ability of the test to correctly identify pregnant animals was (85.7%) on day 7, which means that the incidence of the false-negative results was 14.3%. Similar results were obtained by Cordoba *et al*. (2001) who used the same assay for diagnosing pregnancy in the blood samples of 35 cows collected on day 6 after breeding. The sensitivity was very high (100%) when the ECF test was applied on day 14 post breeding, without obtaining false-negative results (Table 1).The statistical differences between the later two sensitivities were not significant.

Table (1): Number of pregnant and non-pregnant cows as tested positive or negative with ECF, P4 and US tests out of 14 pregnant and 18 non-pregnant cows diagnosed by rectal palpation.

Pregnancy test	Days of gestation	Pregnant cows (14)		Non-pregnant cows (18)	
		A	B	C	D
ECF	7	12	2	14	4
	14	14	0	15	3
P_4	21	14	0	10	8
	24	14	0	12	6
US	30	13	1	18	0

A: True positive results (pregnant). **C: True negative results (non-pregnant).**

B: False negative results (non-pregnant) **D: False positive results (pregnant).**

Regarding to the 18 non-pregnant cows being diagnosed by rectal palpation, it clearly appears that using the ECF test on days 7 and 14 after

breeding suggested that 4 and 3 cows of them, respectively were pregnant. The calculated specificity of the test which measures the ability of the test to correctly identify non-pregnant animals was 77.8% on day 7, increased insignificantly to 83.3% on day 14 after breeding (Table 2), which means that the incidence of the false-positive results decreased from 22.2% on day 7 to 16.7% on day 14 post insemination. The specificity of the ECF test obtained by Cordoba *et al*. (2001) was very low (4%), so the incidence of the false-positive results was markedly very high (96%) than obtained by Des Coteaux *et al*. (2000) or in our study (Table 2). The reason for pregnant cows being diagnosed as non-pregnant could be due to incidence of early embryonic mortality prior to application of transrectal examination. Adams and Jordon, (1999); Des Coteaux *et al*. (2000) attributed the obtained poor accuracy of the ECF test resulted from the high rate of the false-positive results to the occurrence of the embryonic loss during the period of blood samples collection from the cows between 3 to 7 and 11 to 15 days post breeding versus the results of pregnancy diagnosis obtained by either rectal palpation or transrectal ultrasonography carried out between 25 and 60 days post breeding. Similar conclusion was reported by Threlfall, (1994) and Cordoba *et al*. (2001).

Table (2): Sensitivity, Specificity, PPV, NPV and Accuracy of ECF, P4 and US tests.

Pregnancy test	Days of gestation	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
ECF	7	85.7	77.8	75.0	87.5	81.3
	14	100	83.3	82.4	100	90.6
P4	21	100	55.6	63.6	100	75.0
	24	100	66.7	70.0	100	81.3
US	30	92.9	100	100	94.7	96.9

PPV: Positive predictive value. NPV: Negative predictive value.

Data in Table 1 further indicate that the progesterone (P4) assay in the serum samples of cows on day 21 or 24 after breeding was successfully able in detecting all the pregnant cows (which diagnosed pregnant by rectal palpation on day 50 after breeding). So, the sensitivity of the P4 test reached

the maximum (100%) in identifying the pregnant animals. In contrast, the P4 assay failed in detecting the non-pregnant cows with the same sensitivity. Of the 18 cows being diagnosed as non-pregnant by rectal palpation, P4 test conducted on days 21 and 24 post breeding determined 8 and 6 cows of them were pregnant, respectively (Table, 1). Similar results were obtained in goats by Gonzalez *et al*. (2004) in determining pregnant animals, meanwhile the accuracy of identifying non-pregnant animals was comparatively lower (82.8%). The specificity of P4 test was very low on day 21 (55.6%), then it insignificantly increased on day 24 (66.7%) post breeding, indicating that the incidence of the false-positive results was very high (44.4% and 33.4%) on days 21 and 24 after breeding, respectively, which could be attributed to irregular length of estrus cycle, early embryonic mortality, hydrometra or luteal cysts (Pennington *et al*. 1985 and Nebel *et al*. 1987).

The transrectal US was significantly a very effective method in detecting all the non-pregnant cows. This method determined one cow of the 14 cows diagnosed pregnant by palpation was non-pregnant. The calculated sensitivity (92.9%) was lower than that recorded by Pieterse *et al*. (1990) and Nation *et al*. (2003) (97.7% and 96%, respectively). However, the obtained specificity (100%, Table 2) was higher than that reported by them (87.7% and 97%, respectively).

Data in Table 2 indicate that the PPV for ECF test was lower on day 7 and day 14 after breeding (75% and 82.4%, respectively) than that of NPV (87.5% and 100%, respectively). The same trend was true for the P4 test either on day 21 or 24 after breeding. It means that the probability of diagnosing cow as truly pregnant was lower than that of diagnosing cow as truly non-pregnant for both tests. The probability of diagnosing cows truly open was 100% (NPV) when conducting ECF test on day 14 or P4 test either on day 21 or 24 after breeding. The opposite trend was true when using US where the PPV was higher (100%) than the NPV (94.7%).

The transrectal US method proved the highest (96.9%) accuracy in diagnosing that cow accurately (regardless result of diagnosing pregnant or open) than that ECF test (81.3% and 90.6% on days 7 and 14 after breeding, respectively) and that P4 test (75% and 81.3% on days 21 and 24 after breeding, respectively).

From the foregoing results, it could be concluded that the sensitivity of the ECF, P4 and US techniques for pregnancy diagnosis in dairy cattle was 100, 100 and 92.5% on days 14, 21-24 and 30 after breeding, respectively (Table 2). The specificity for the same procedures were 83.3, 55.6-66.7 and 100%, meanwhile the PPV were 82.4, 63.6-70.0 and 100%, the NPV were 100,

100 and 94.7% and the accuracy were 90,6, 75.0-81.3 and 96.9%, respectively (Table 2). Moreover, Using ECF test on day 14 after A.I seems to be more effective than that on day 7 after A.I. However, both of them could give reliable early information about pregnancy diagnosis than the other tested methods with an accuracy of 81.3-90.6% and could be conducted as a valuable method in culling and rebreeding of non-pregnant cows besides being as a valuable tool for controlling breeding program.

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دراسة مقارنة لتشخيص الحمل بواسطة استخدام اختبار عامل الحمل المبكر، مستوى البروجسترون وجهاز السونار المستقيمى فى الأبقار

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الملخص العربى

كان الهدف من هذه الدراسة هو مقارنة استخدام طريقة اختبار الحمل المبكر ، وطريقة تقدير مستوى البروجسترون فى سيرم الدم وكذلك طريقة استخدام السونار المستقيمى للكشف عن الحمل فى الأبقار الحلابة. استخدم عدد ٣٢ بقرة هولستين يتراوح أعمارها من ٤-٨ أعوام. تم جمع عينات الدم من كل بقرة فى الأيام ٧ ، ١٤ بعد التلقيح لإجراء إختبار عامل الحمل المبكر وكذلك فى الأيام ٢١ و ٢٤ بعد التلقيح لتقدير مستوى البروجسترون فى سيرم الدم. كما تم استخدام طريقة السونار المستقيمى لتشخيص الحمل فى اليوم الـ ٣٠ بعد التلقيح . تم تقييم هذه الاختبارات ومقارنتها بنتائج اختبار تشخيص الحمل بطريقة الجس المستقيمى فى اليوم ٥٠ بعد التلقيح. كما تم حساب درجة حساسية ودقة الإختبارات الثلاثة في الكشف عن الحيوانات الحامل والحيوانات الغير حامل .

أوضحت النتائج أن استخدام طريقة عامل الحمل المبكر فى اليوم السابع بعد التلقيح أظهر وجود بقرتان غير حامل من جملة ١٤ بقرة (تم تشخيصهم أنها حوامل بواسطة طريقة الجس المستقيمى في اليوم ٥٠ بعد التلقيح) . بينما استخدام طريقة عامل الحمل المبكر فى اليوم الرابع عشر بعد التلقيح كان قادرا على اكتشاف جميع الحيوانات الحامل. كانت درجة حساسية الاختبار فى اليوم السابع ٨٥.٧% ، ارتفعت إلى١٠٠% فى اليوم ١٤ بعد التلقيح. وكان معدل حدوث النتائج السلبية الغير صحيحة ١٤.٣% ، صفر % في الأيام ٧ و ١٤ بعد التلقيح ، على التوالى . بالنسبة لـ ١٨ بقرة والتى تم تشخيصها غير حامل بواسطة طريقة الجس

المستقيمى فقد أظهر إجراء اختبار عامل الحمل المبكر في اليوم السابع والرابع عشر بعد التلقيح أن هناك ٤ و٣ بقرات منهم غير حامل ، على التوالى. وكانت درجة حساسية الاختبار فى الكشف عن الحيوانات الغير حامل ٧٧.٦% فى اليوم السابع ، تحسنت إلى ٨٣.٣% فى اليوم ١٤ بعد التلقيح وذلك مقارنة بطريقة الجس المستقيمى . وانخفض معدل حدوث النتائج الإيجابية الغير صحيحة من ٢٢.٢% فى اليوم السابع إلى ١٦.٧% فى اليوم ١٤ بعد التلقيح.

كما أوضحت النتائج أن استخدام طريقة اختبارالبروجسترون فى يوم ٢١ أو ٢٤ بعد التلقيح كان ناجحاً فى التعرف على جميع الأبقار الحامل التى تم تشخيصها بواسطة طريقة الجس المستقيمى. وكانت درجة حساسية إختبار البروجسترون ١٠٠% في التعرف على الحيوانات الحامل. بينما لم يستطيع إختبار البروجسترون التعرف على الأبقار الغير الحامل بنفس المستوى. وكانت درجة حساسية الاختبار فى اكتشاف الأبقار الغير الحامل منخفضة فى اليوم ٢١ (٥٥.٦%) عن اليوم ٢٤ (٦٦.٧%) بعد التلقيح. مما أدى إلى إرتفاع معدل حدوث النتائج الإيجابية الغير صحيحة في اليوم ٢١ (٤٤.٤%) واليوم ٢٤ (٣٣.٣%) بعد التلقيح. كان استخدام طريقة السونار المستقيمى ناجحا بدرجة عالية (١٠٠%) فى اكتشاف جميع الأبقار الغير الحامل . يستخلص من هذه الدراسة أن استخدام اختبار الحمل المبكر فى اليوم ١٤ بعد التلقيح كان أكثر كفاءة فى تشخيص الحمل عن استخدامه فى اليوم السابع بعد التلقيح بدرجة دقة ٨١.٣ . ٩٠.٦% ، وبالتالى فإن استخدام هذه الطريقة في تشخيص الحمل في الأبقار قد تكون طريقة فعالة فى معرفة وإعادة تلقيح الحيوانات الغير حامل فى وقت مبكر عن طرق التشخيص الأخرى بجانب أنها تعتبر أداه ذات قيمة فى تنفيذ برامج التربية .