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THE ACCURACY OF MILK PROGESTERONE ASSAY FOR EARLY DIAGNOSIS OF PREGNANCY IN DAIRY COWS AND BUFFALOES

(With 2 Tables)

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مدى دقة تقدير البروجستيرون في اللبن في تشخيص الحمل
المبكر في الأبقار الحلوب والجامـــــــــــوس

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تضمن البحث تحديد مدى دقة البروجستيرون في اللبن في التشخيص المبكـــــر للحمل في الأبقار الحلوب وإناث الجاموس وذلك بإستخدام طريقة قياس الإشعـــــــاع المناعي في تحديد تركيز هرمون البروجستيرون في لبن هذه الحيوانات بعد مـــــــــرور ٢٤ يوما على تلقيحها . وقد تم الإعتماد في ذلك على تأكيد حدوث الحمل من خـــــلال الفحص عن طريق المستقيم لهذه الحيوانات بعد إنقضاء شهرين على تلقيحها . أظهـــــرت النتائج أنه في حالة الحيوانات غير العشار فإن دقة هذا الإختبار كانت ١٠٠٪ أما فـــي حالة الحيوانات العشار فقد كانت النسبة ٧ر٨٥٪ و ٥ر٨٧٪ في الأبقار والجاموس علـــــى التوالي . كما أن مستوى هرمون البروجستيرون في لبن الأبقار العشار قد تراوح بيـــــن أكثر من ٢ نانوجرام وحتى ١٣ نانوجرام / مل وفي حالة الجاموس تراوح مستوى الهرمون بين أكثر من ١ نانوجرام ، ٩ نانوجرام / مل . تضمن البحث أيضا مناقشة الإعتبـــــارات المختلفة الواجب مراعاتها عند تحليل نتائج هذا الإختبار .

SUMMARY

The accuracy of milk progesterone assay for the early diagnosis of pregnancy in dairy cows and buffalo-cows was assessed using a radio-immunoassay technique for determination of progesterone concentration in the whole milk of those animals 24 days after service. This was based upon the subsequent confirmation of occurrence of pregnancy by rectal examination 2 months after breeding. In case of non-pregnancy, the hormone assay was 100% accurate. The efficiency of the test in identifying pregnant cows and buffalo-cows was 85.7% and 87.5% with progesterone concentrations ranged from > 2 to 13 ng/ml and > 1 to 9 ng/ml of milk of both animals, respectively. Different consider-ations in the interpretation of the test results were also discussed.

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INTRODUCTION

The use of milk progesterone assay for pregnancy diagnosis and for fertility monitoring among dairy cows was first described by HEAP et al. (1973). Since that time, different techniques have been described by several authors allover the world for determination of milk progesterone concentration either in specific separated fractions of milk (HOFFMAN et al., 1976 and BOOTH et al., 1979) or in whole milk (HOLDSWORTH et al., 1979; SALEH, 1985 and REIMERS et al., 1990). The accuracy of the assay for diagnosis of pregnant and non-pregnant cows 20-25 days after insemination was 80 - 85% and 95%, as it was recorded by HEAP et al. (1973) and HOFFMAN and HAMBURGER (1973), respectively. HOLDSWORTH et al. (1980) recorded an accuracy of 96.7% and 100% for pregnant and non-pregnant cows, respectively. GOWAN et al. (1982) revealed that the accuracy of the assay was 76.9% and 93.8% for diagnosis of the two categories of cows, respectively.

Concerning the concentration of progesterone hormone in milk of inseminated cows 20-25 days after insemination, SCHIAVO et al. (1975) reported that it averaged 7.12 ng/ml in pregnant and 2.36 ng/ml in non-pregnant cows. HOFFMAN et al. (1976) revealed that cows with concentrations less than 2 ng/ml of progesterone in milk, were non-pregnant. HEAP et al. (1976) recorded that the mean progesterone concentration in milk 21 and 24 days after insemination was 34.7 ± 1.7 and 35.0 ± 1.7 ng/ml in pregnant cows and it was 15.4 ± 2.3 and 22.7 ± 3.3 ng/ml in non-pregnant cows, respectively. BOOTH et al. (1979) and HOLDSWORTH et al. (1980) mentioned that milk progesterone levels below 5.5 ng/ml were considered as indication of non-pregnancy and that cows having more than 7.5 ng/ml of the hormone in their milk were considered as pregnant.

Concerning progesterone assay in the milk of buffalo-cows, MURRAY et al. (1990) determined the hormone concentrations in the milk of Murrah buffaloes during different phases of oestrus cycle. They revealed that the milk of those animals contained < 1 ng/ml of progesterone hormone during estrus phase and upto 14 ng/ml during luteal phase. ISMAIL et al. (1991) used a radioimmunoassay technique for determination of progesterone in the milk of Egyptian buffaloes to evaluate their reproductive performance. They found that the hormone concentration was 3.9 ± 0.2 ng/ml in case of pregnancy and luteal phase and it was $\angle 1.0$ ng/ml in case of estrus or inactive ovaries.

The present work was carried out to detect the accuracy of progesterone assay in the whole milk for the early diagnosis of pregnancy in dairy cows and buffalo-cows 24 days after breeding.

MATERIAL and METHODS

A total of 40 Friesian dairy cows and 40 Egyptian buffalo-cows were included in the present work and they were kept in two of the dairy farms belonging to Assiut Governorate Animal Wealth Project. All animals were subjected to careful gynaecological examination for soundness of genital organs and for normal cyclical changes.

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They were kept under close observation for estrus detection and they were naturally-bred twice, once at the middle of heat and the second mating was shortly before the end of heat, using fertile bulls and the breeding date was recorded for each animal. Milk samples were collected in sterile screw-capped glass bottles from the bulk yield of each cows at 8 A.M. (morning-milking) on the 24th day after the recorded breeding date. The collected milk samples were put in a thermos flask containing ice-cubes and taken to the laboratory, where sodium azide was added to them (25 ul, 20% w/v) and they were kept at -20°C until the assay of proesterone hormone. Hormonal assay was carried out using progesterone RIA Kit supplied by the International Atomic Energy Agency, Vienna, Austria following the techniques of the manufacturer and HOLDSWORTH et al. (1979). Rectal examination was carried out 2 months after the recorded breeding date of each cow for pregnancy diagnosis. The accuracy of the hormone assay was assessed on the basis of the result of pregnancy diagnosis by rectal examination.

RESULTS

The results of progesterone assay in milk of the examined cows and buffalo-cows, together with the rectal findings, 2 months after service are presented in tables (1 & 2), respectively.

Five out of the 40 examined dairy cows were having less than 2 ng/ml of progesterone hormone in their milk and they returned to estrus 3 days before sampling. The hormone concentration in the milk of the remaining 35 cows was more than 2 ng/ml. Thirty (85.7%) of them were proved to be pregnant on rectal palpation 2 months after service, while 5(14.3%) were non-pregnant with prominent corpora lutea in their ovaries and without palpable abnormal changes in their genital organs.

Out of the 40 milk samples collected from the examined buffalo-cows (Table 2), 8 samples contained less than 1 ng/ml of progesterone. Six of those animals returned to estrus 48 hours before sampling but the other 2 buffalo-cows did not show signs of heat, however they were diagnosed non-pregnant later on. The remaining 32 milk samples contained more than 1 ng/ml of progesterone. Pregnancy diagnosis by rectal palpation, 2 months after service, revealed that 28(87.5%) of those buffalo-cows were pregnant and 4(12.5%) were non-pregnant, however they had corpora lutea in their ovaries together with palpable metritis in 2 of them.

DISCUSSION

An important feature of the bovine oestrus cycle is the abrupt fall in peripheral plasma progesterone levels which occurs between the third and second day before estrus. Since this fall is not seen in pregnant animals, an early pregnancy diagnosis can be made successfully at this time by progesterone determination in plasma as well as in milk (HEAP et al., 1973; HOLDSWORTH et al., 1979; SHEMESH et al., 1983;

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JANOWSKI, 1988 and DIONYSIUS, 1991). The results of the present work revealed that determination of progesterone hormone in the whole milk of dairy cows, 24 days after service was 100% diagnostic in case of non-pregnancy. The accuracy of the assay for pregnancy diagnosis was 85.7% compared with rectal palpation, carried out 2 months after service. Nearly similar accuracies were previously recorded by HEAP et al. (1973); HOFFMAN & HAMBURGER (1973); JANOWSKI (1988) and REIMERS et al. (1990). However, higher accuracy (96.7% and 100%) was obtained by HOLDSWORTH et al. (1980) and lower accuracies (76.9% and 93.8%) were recorded by GOWAN et al. (1982) and (66-68% and 90-91%) by DIONYSIUS (1991) in terms of identifying pregnant and non-pregnant dairy cows, respectively.

Moreover, the obtained results revealed that the hormone level in the milk of pregnant cows ranged from $>$ 2 ng/ml to 13 ng/ml, which coincided with the hormone levels previously recorded by HOFFMAN et al. (1976) who revealed that the concentration of progesterone hormone in the milk of pregnant cows, 20 days after insemination, was more than 2 ng/ml. REIMERS et al. (1980) found that 0%, 50% and 83% of tested cows were pregnant by rectal palpation when their milk contained $<$ 1.0, 1-3 and $>$ 3 ng/ml of progesterone hormone, respectively 21-24 days after breeding. Moreover, nearly similar milk progesterone levels were also detected in pregnant cows by RAINIO (1987); JANOWSKI (1988) and REIMERS et al. (1990). On the other hand, higher levels of the hormone were estimated in the milk of pregnant cows by HEAP et al. (1976); COX et al. (1978) and DIMITROV et al. (1989).

These differences in the accurcay of milk progesterone assay for the early diagnosis of pregnancy as well as the hormone concentration in the milk of the examined cows and those recorded by other workers, might be attributed to the different techniques used for hormonal assay. The breed of the examined animals as well as the efficiency of their corpora lutea in secreting progesterone and the level of other steroids that might be dissiminated in their milk, should be also considered. Moreover, FAYEMI et al. (1982) revealed that there was a significant increase in the concentration of progesterone in milk containing Staphylococcus aureus organisms collected from cows having subclinical mastitis. This should be also considered in the interpretation of the results of progesterone assay.

The accuracy of progesterone assay in identifying pregnant and non-pregnant buffalo-cows, 24 days after breeding, was 87.5% and 100% for the two categories of animals, respectively. Moreover, the concentration of the hormone in the milk of pregnant animals ranged from 1.1 to 9 ng/ml, which is lower than its concentration in the milk of pregnant cows. Nearly similar results were reported by MURRAY et al. (1990) and ISMAIL et al. (1991). The relatively smaller corpus luteum of buffalo-cows and consequently its leser efficiency in secretion of progesterone should be taken into consideration.

Finally, it can be concluded that milk progesterone assay can be used efficiently in the early monitoring, of conception in both cows and buffalo-cows. However, care

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should be taken in case of milk samples containing increased count of mastitis bacteria. This should be confirmed by rectal palpation 5-6 weeks later to minimize economic losses arising form misdiagnosis in case of non-pregnancy with or without pathological affections in the reproductive organs of the examined animals.

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Table (1) Results of progesterone assay and Rectal examination
of the examined cows

No of examined cows	Progesterone level ng/ml milk	Rectal Finding		Accuracy of progesterone assay
		Pregnant	Non-preg	
2	0-1	-	2	100 %
3	1.1-2	-	3	100 %
11	2.1-3	9	2	81.82%
5	3.1-4	4	1	80.0 %
6	4.1-5	5	1	83.33%
4	5.1-6	3	1	75.0 %
2	6.1-7	2	-	100 %
1	7.1-8	1	-	100 %
3	8.1-9	3	-	100 %
-	9.1-10	-	-	-
-	10.1-11	-	-	
2	11.1-12	2	-	100 %
1	12.1-13	1	-	100 %

Table (2) Results of progesterone assay and Rectal examination
of the examined buffalo-cows.

No of examined buffalo-cows	Progesterone level ng/ml milk	Rectal Findings		Accuracy of progesterone Assay
		Pregnant	Non-pregnant	
8	0 - 1	-	8	100 %
12	1.1- 2	9	3	75.0%
4	2.1- 3	3	1	75.0%
2	3.1- 4	2	-	100 %
2	4.1- 5	2	-	100 %
3	5.1- 6	3	-	100 %
5	6.1- 7	5	-	100 %
2	7.1- 8	2	-	100 %
2	8.1- 9	2	-	100 %