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USE OF MILK PROGESTERONE ASSAY FOR MONITORING OVULATION, OVARIAN CYCLES AND PREGNANCY IN BUFFALO

Osman, K. T.[*]; H. A. El-Regalaty[*]; A.E. Fekry[**]; H. M. Farghaly[**] and H.B. Aboul-Ela[*]

[*] **Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture.**

[**] **Nuclear Research Center, Atomic Energy Authority.**

ABSTRACT

This work aimed at investigating the relationship between progesterone (P_4) concentrations in milk and blood in dairy buffalo. The reliability of using milk P_4 level as an easy diagnostic tool for monitoring ovarian cycles, ovarian dysfunction and pregnancy was investigated. Twenty buffalo cows (Ten pregnant and 10 non-pregnant) were used. Four hundred sixty blood samples and another 460 milk samples were analyzed for P_4 assay using RIA technique. Different correlation coefficients between P_4 levels in milk and blood serum were computed. The P_4 profiles for the different physiological cases of the animals were diagrammed.

The results showed that P4 concentrations were consistently higher ($p<0.01$) in almost all milk samples matched to those of blood serum. The P_4 levels in pregnant buffalo ranged from 2.0-8.5 ng/ml in serum and from 3.1-18.6 ng/ml in milk. The P_4 levels in non-pregnant animals ranged from 0.1-8.5 ng/ml in serum and from 0.1-19.9 ng/ml in milk. The differences in P_4 levels between pregnant and non-pregnant animals, as well as between milk and serum within each group were statistically significant ($p<0.01$). The overall mean milk P_4 level in pregnant buffalo (11.3 ±0.2 ng/ml) was 2.3 times higher than that of blood serum (4.9±0.1 ng/ml). The milk P_4 level in non- pregnant buffalo (6.8± 0.5 ng/ml) was 2.5 times higher than that of the serum (2.7±0.2 ng/ml).

Milk and serum P_4 concentrations correlated significantly ($p<0.001$) in both pregnant ($r = 0.50$) and non-pregnant ($r = 0.87$) buffalo. The total correlation coefficient (considering all samples from both pregnant and non-pregnant animals) was 0.83 ($p<0.001$).

The overall mean ovarian cycle length was 20.2 ± 1.5 days with a range of 12-42 days. Half of the cycles (50%) had normal length (18-24 days). Percentages of short (≤ 17 days) and long (≥25 days) cycles were 31.8 and 18.2%, respectively. All ovulations were confined to P_4 levels ranging from 0.1- 2.9 ng/ml in milk and from 0.1-0.4 ng/ml in serum. The P_4 peak levels of the mid ovarian cycle ranged from 6.7-17.8 ng/ml in milk and from 2.6-8.5 ng/ml in serum. It was concluded that an abrupt decline in milk P_4 level to ≤ 2.9 ng/ml in regularly monitored buffalo could be useful indicator for the occurrence of ovulation. Confirming ovulation on the basis of a single milk sample could be misleading as the accuracy of diagnosis may be interrupted by the possibly encountered cases of static inactive ovaries or ovarian follicular cysts. The P_4 assay in repeated milk samples can provide a wider vision for accurate diagnosis of the case.

It was shown that all pregnant buffalo had P_4 levels > 3.0 ng/ml in milk. In addition, all buffalo cows with milk P_4 levels of < 3.0 ng/ml were non-pregnant. That is to conclude that dairy buffalo with < 3.0 ng/ml P_4 level in milk could be diagnosed non-pregnant with high degree of accuracy. Otherwise, the use of P_4 level of > 3.0 ng/ml in milk could only be suggestive for the occurrence of pregnancy. The possibly encountered cases of ovarian luteal cysts or persistent CLs could interrupt the accuracy of diagnosis.

It was concluded that milk P_4 assay could be used as an efficient mean for diagnosing non-pregnant buffalo with high degree of accuracy. Otherwise, it is tentatively suggestive for diagnosing pregnant buffalo. The milk P_4 assay could also be an easy and useful mean for monitoring ovulation, ovarian dysfunction, embryonic mortality and pregnancy in buffalo, especially under rural conditions.
Keywords: Buffalo, Progesterone profiles, Milk, Blood, Ovarian cycles, Ovarian dysfunction, Pregnancy.

INTRODUCTION

Buffalo occupy a prominent position in animal agriculture system in Egypt. Beside to their importance as main dairy producers, they secondarily contribute to a considerable bit of the total national red meat production. Despite their importance, buffalo have been traditionally accused to have lower reproductive capacity compared with *Bos-taurus* cattle. Their long generation interval (Metry *et al.*, 1994) due to long calving intervals (Singh, *et al.*, 2000) has been regarded as a fundamental obstacle retarding genetic improvement in this animal.

It should be mentioned that long calving interval in buffalo is more pronounced on the side of small farmers rather than at the level of commercial herds (Osman, 2005). Factors affecting calving interval in buffalo involve female-side reproductive problems and management.

It has been well established that corpus luteum (CL), the transient endocrine structure on the ovary, plays a pivotal role in controlling reproductive cycles in mammals (Niswender *et al.*, 2000 and Diaz *et al.*, 2002). Progesterone (The main steroid secreted by the CL) in either milk or blood has long been reported to provide good estimate of luteal function in female farm animals (Kamboj and Prakash, 1993; Ucar *et al.*, 2004 and Garmo, *et al.*, 2009). It has also been reported that P_4 concentrations in both blood and milk are related to each other with similar or higher P_4 levels in milk (Heap, *et al.*, 1974.; Ginther *et al.*, 1976 and Cox, *et al.*, 1987). These authors mentioned that P_4 levels in milk tended to reflect their levels in blood and that milk P_4 levels could be useful indicators for confirming estrus and diagnosing pregnancy in cattle.

Ucar *et al.* (2004) suggested that accurate determination of milk P_4 could be used as an easy and useful mean for monitoring estrus, ovarian dysfunction, embryonic mortality and pregnancy in buffalo, especially under rural conditions. In the same context, collection of milk samples for P_4 assay was found to be more agreed by the farmers rather than collecting blood samples (Kamboj and Prakash, 1993 and Qureshi, *et al.*, 2000). These farmers have the belief that collecting blood samples is invasive to their animals and adversely affect milk production, estrous manifestation and appetite.

This work aimed at investigating the relationship between milk and blood P_4 levels in buffalo. The reliability of using milk P_4 assay as a diagnostic tool for monitoring ovulation, ovarian dysfunction and pregnancy in buffalo was also investigated.

MATERIALS AND METHODS

Location of the farm

This study was conducted at Mehallet Moussa Research Station, Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture. The farm is located in the North center of Nile Delta, Kafr El-Sheikh Governorate.

Experimental animals

Twenty clinically normal, lactating buffalo cows (Ten pregnant and 10 non-pregnant) were used in this study. They ranged in their ages between 4 - 12 years and parities between the 2^{nd} and 7^{th} lactations. The animals were kept under the ordinary management conditions applied on the farm. The feeding rations (comprising pelleted concentrate mixture, berseem hay and wheat straw) were calculated on the basis of body weight, milk production and physiological status of the dams (APRI, 1997). Milking of the dams was practiced twice daily at 7 am and 4 pm.

Milk and blood sampling

A total of 460 milk-samples and another 460 peripheral-blood samples (230 samples of each sample-type from each group) were obtained. The field-sampling period was extended from May to July 2007. During this period, breeding of non-pregnant buffalo (n=10) was avoided. Milk samples of 15 ml/animal were collected at 4-days intervals during the 7-am milking. Blood samples were collected at the rate of 10 ml/animal on the same days of milk sampling. Using the puncture technique, blood samples were drained from the jugular vein into evacuated glass tubes. Blood and milk samples were then centrifuged at 1500g, blood sera and fat- free milk separated and kept frozen at –20°C until the analysis for P_4 was performed.

Progesterone assay

Assessment of P_4 concentration in fat free milk and blood serum was performed by radio-immunoassay technique. A commercial pre-coated antibody tube kits (Diagnostic Products Corporation, Los Angeles, CA, USA.) were used. The assay sensitivity was 0.10 ng/ml. The intra and inter-assay variation coefficients were 6.9 and 10.1%, respectively.

Ovarian cycles

The ovarian cycle was defined as the time elapsed between two consecutive ovulations that is concomitant to a P_4 basal level of < 1.0 ng/ml in blood serum, followed by a sustained level of $\geq$ 1.0 ng/ml until the subsequent abrupt decline in its level to < 1.0 ng/ml. A total of 22 complete ovarian cycles displayed by the females in the non-pregnant group, were investigated. The incomplete cycles (That started or ended outside the field-sampling period) were ignored. The ovarian cycles were classified into normal (18-24 days), short ($\leq$ 17 days) and long ($\geq$ 25 days). The incidence of each cycle type was calculated.

Progesterone profiles

The P_4 profiles representing the different physiological cases of the experimental buffalo were scrutinized. The results of P_4 profiles were discussed and plotted in diagrams for each individual animal.

Statistical analysis

The statistical analysis was performed using SAS computer program. The overall means ±SE for the different P_4 levels studied were computed. The analysis of variance was computed using the general linear models procedure of SAS (GLM/SAS, 2002). The simple Pearson correlation coefficients between milk and serum P_4 levels in pregnant and non-pregnant cows were also computed. The incidence of the different types of ovarian cycles was calculated in percentages.

RESULTS AND DISCUSSION

Milk and serum P_4 levels in pregnant and non-pregnant buffalo

The overall mean P_4 concentrations in milk and serum of pregnant and non-pregnant buffalo cows are shown in table 1.

It was not new to find that the P_4 concentrations in both milk and serum were significantly higher ($p<0.01$) in pregnant than in non-pregnant animals. It was also shown that P_4 concentrations in almost all milk samples were consistently higher ($p<0.01$) than their corresponsive levels in blood serum (Kamboj and Prakash, 1993; Qureshi, *et al.*, 2000 and Ucar, *et al.*, 2004). The serum P_4 concentration in pregnant animals ranged from 2.0-8.5 ng/ml with an overall mean of 4.9±0.1ng/ml. On the other hand, it ranged from 3.1 to 18.6 ng/ml with an overall mean of 11.3±0.2 ng/ml in milk. The corresponding values in non-pregnant buffalo serum ranged from 0.1-8.5 ng/ml with an overall mean of 2.7±0.2ng/ml and from 0.1-19.9 ng/ml with an overall mean of 6.8±0.5 ng/ml in milk. The differences in P_4 concentrations between both groups, as well as within each group were statistically significant ($p<0.01$).

Table 1: Serum and milk P_4 levels (ng/ml) in pregnant and non-pregnant buffalo cows.

Item	Pregnant		Non-pregnant	
	Mean±S.E.	Range	Mean±S.E.	Range
Serum P_4 (ng/ml)	4.9 ± 0.1^{a} (230)	2.0 - 8.5	2.7 ± 0.2^{b} (230)	0.1 - 8.5
Milk P_4 (ng/ml)	11.3 ± 0.2^{a} (230)	3.1-18.6	6.8 ± 0.5^{b} (230)	0.1-19.9

Means with different superscripts in the same row differ significantly ($p<0.01$).
Figures in parenthesis indicate the number of observations.

Milk/serum P_4 ratio and coefficients

As shown in table 2, the overall mean milk P_4 concentration in pregnant buffalo was 2.3 times higher than that of blood serum. In the same context, the overall mean milk P_4 level in non- pregnant buffalo was 2.5 times higher than that of blood serum. Similar trends, but higher milk/serum P_4 ratios were early reported by Ginther *et al.* (1976) in cattle and Batra *et al.* (1979) in buffalo. They found that P_4 concentration in milk was four to five times higher than that of blood plasma.

It was worth to mention that milk and serum P_4 concentrations were significantly ($p<0.001$) correlated in both, pregnant ($r=0.50$) and non–pregnant ($r=0.87$) buffalo (Table 2). The total correlation coefficient (considering all samples from both pregnant and non-pregnant animals) was 0.83 ($p<0.001$).

Table 2: Milk/serum P4 ratios and correlation coefficients between milk and serum P4 levels in pregnant and non-pregnant buffalo cows.

Item	Pregnant	Non-pregnant	Total
Milk/serum P4 ratio	2.3 : 1 (460)	2.5 : 1 (460)	2.4 : 1 (920)
Correlation coefficient	0.50 (460)	0.87 (460)	0.83 (920)

Figures in parenthesis indicate the number of observations.

These results came in almost complete agreement with those reported by Kamboj and Prakash (1993) who recorded correlation values ranging from 0.82 to 0.89 between milk and blood P_4 levels in cyclic buffalo. Higher correlations of 0.98 and 0.99 were reported by Batra *et al.* (1979) for pregnant and non-pregnant buffalo, respectively.

It was not clear whether the consistently higher P_4 levels in milk compared to serum were due to transfer of P_4 from blood to milk or due to the synthesis of P_4 by the mammary tissue (Heap *et al.*, 1975). The later authors suggested that the presence of P_4 in cow milk was attributed to its ability to diffuse against concentration gradients from blood to milk. They concluded that high P_4 levels measured in milk could be a result of lipid solubility of steroids. However, there is evidence for synthesis of P_4 in the mammary gland in goat from infusion of pregnenolone to the gland (Slotin *et al.*, 1970).

Progesterone profiles of individual animals

Progesterone profiles representing the different physiological patterns of buffalo cows are illustrated in plates 1 and 2.

As shown in the plates, the experimental buffalo could be grouped as pregnant (Animals from 11 - 20), cyclic (Animals no. 2, 3, 5, 7, 9 and 10), totally acyclic (Animal no. 8) and those apparently restoring cyclicity after long periods of anestrum (Animals no. 1, 4 and 6). In pregnant group, the P_4 concentrations ranged from 2.0-8.5 ng/ml in blood and from 3.1- 18.6 ng/ml in milk. In the cyclic group, the animals showed almost normal P_4 profiles except in animals 5, 9, 10 (plate 1) as they displayed $\geq$ one short or long P_4 cycles interrupting the normal cycles. It was also seen that the ovarian activity in animal no. 8 was totally lacking. In this animal, the P_4 concentrations in both milk and serum were almost undetectable and remained at their basal levels throughout the period of field sampling. Animals no. 1, 4, 6 underwent prolonged periods of anestrum (plate 1). Animal no. 4 showed a prolonged period of ovarian inactivity. During this period, the P_4 levels were at their basal limits. This was followed by a short duration P_4 cycle threatening the onset of cyclicity. In animals 1 and 6 the continued elevation in P_4 levels during the periods of anestrum may reflect the occurrence of a pregnancy

that is followed by embryonic loss (Samad, *et al.*, 2004 and Osman, *et al.*, 2005). Nevertheless, this may also be attributed to the presence of a persistent lutienized structure on the ovary that undergoes subsequent auto-recovery before restoring cyclicity (Qureshi, *et al.*, 2 000).

In non-pregnant buffalo, the highly significant ($p<0.001$) correlation coefficient between P_4 levels in milk and serum ($r=0.87$) suggests that P_4 determination in milk could be useful indicator for diagnosing ovarian dysfunction in buffalo especially if assessed in repeated consecutive samples (Samad, *et al.*, 2004 and Ucar, *et al.*, 2004).

Ovarian cycles

The length and incidence of the different types of ovarian cycles are shown in table 3.

The overall mean ovarian cycle length was 20.2 ± 1.5 days with a range of 12-42 days. It was shown that 50% of the cycles had normal length (18-24 days). On the other hand, the percentages of the short (≤ 17 days) and long (≥ 25 days) cycles were 31.8 and 18.2%, respectively. These results came in a partial agreement with the early findings of Hafez (1954) and El-Nouty (1971). On the other hand, they were closer to the results of Barkawi and Aboul Ela (1987) and almost similar to those reported by El-Terbany (1998) and Osman (2005). Conversely, the current results disagreed with those of Mohamed, *et al.*, (1974) who reported an incidence of 28% for long ovulation cycles (≥ 46 days) in post-partum buffalo cows. In fact, this high incidence of long ovulation cycles may involve high incidence of unreal cycles and could rather be attributed to either embryonic mortality (Hafez, 1954 and El-Nouty, 1971 and Osman, *et-al.*, 2005) or inefficient heat detection measures (Osman, 2005).

Table 3: Length and incidence of the different types of ovarian cycle patterns in buffalo cows.

Ovarian cycle pattern	Normal (18-24 days)	Long (≥25 days)	Short (≤ 17 days)	Overall total
Mean length ± SE (Days)	21.1±0.6 (11)	30.5±4.0 (4)	12.7±0.9 (7)	20.2±1.5 (22)
Recorded range (Days)	18 – 24	25 – 42	12 – 17	12 – 42
Incidence	50 %	18.2 %	31.8 %	100 %

Figures in parenthesis indicate the number of cycles.

The P_4 level on the day of ovulation, as well as of the peak of the mid-ovarian cycle in both serum and milk are shown in table 4 and plotted in plate 1.

It could be seen from the table that all ovulations were confined to P_4 levels ranging from 0.1- 2.9 ng/ml in milk. The corresponding levels in the blood serum ranged from 0.1-0.4 ng/ml.

It was also shown that the P_4 peak levels in milk during the mid ovarian cycle ranged between 6.7-17.8 ng/ml. The concomitant P_4 levels in the serum ranged from 2.6-8.5 ng/ml. These ranges came in a good harmony with those reported by Ucar, *et al.*, (2004) and Osman (2005) during the same stage in milk and blood, respectively.

Table 4: Blood serum and milk P4 concentrations (ng/ml) in relation to some physiological events of the ovulation cycle in buffalo.

Progesterone concentration (ng/ml)	Serum		Milk	
	Mean ± SE	Range	Mean ± SE	Range
On the day of ovulation	0.15 ± 0.01 (33)	0.1 - 0.4	0.6 ± 0.1 (33)	0.1 - 2.9
Peak of mid ovulation cycle	6.1 ± 0.3 (22)	2.6 - 8.5	13.3 ± 0.6 (22)	6.7 - 17.8

Figures in parenthesis indicate the number of observations.

Reviewing the literature, it could be seen that the previous results on the use of milk P_4 level for monitoring reproductive performance in buffalo are widely variable (Batra *et al.*, 1979; Kamboj and Prakash, 1993 and Ucar, *et al.*, 2004). This variability could possibly be attributed to either variability in estrous-cycle patterns (Gupta and Prakash, 1990) or in milk-fat content (Qureshi, *et al.*, 2000). In the current study, the comparatively narrow variability in the ovarian-cycle patterns and lack of influence of milk-fat (due to use of fat-free milk) have led to more reliable results compared to those reported in the literature.

Concluding remarks

In this study, characterization of the ovarian cycles involves an overall mean cycle length of 20.2±1.5 days, P_4 levels in milk and serum of 0.6±0.1 & 0.15±0.01 ng/ml on the day of ovulation and peaks of 13.3±0.6 & 6.1± 0.3 ng/ml during the mid ovarian cycle, respectively.

It has been shown that all ovulations in this study are confined to P_4 levels ranging from 0.1- 2.9 ng/ml in milk and from 0.1- 0.4 ng/ml in serum. Hence, it could be suggested that an abrupt decline in milk P_4 level to $\leq$ 2.9 ng/ml in regularly monitored buffalo could be useful indicator for the occurrence of ovulation. Confirming ovulation on the basis of a single milk sample could be misleading as the accuracy of diagnosis may be interrupted by cases of static inactive ovaries or ovarian follicular cysts. Thus, the P_4 assay in repeated milk samples can provide a wider vision for accurate diagnosis of the case.

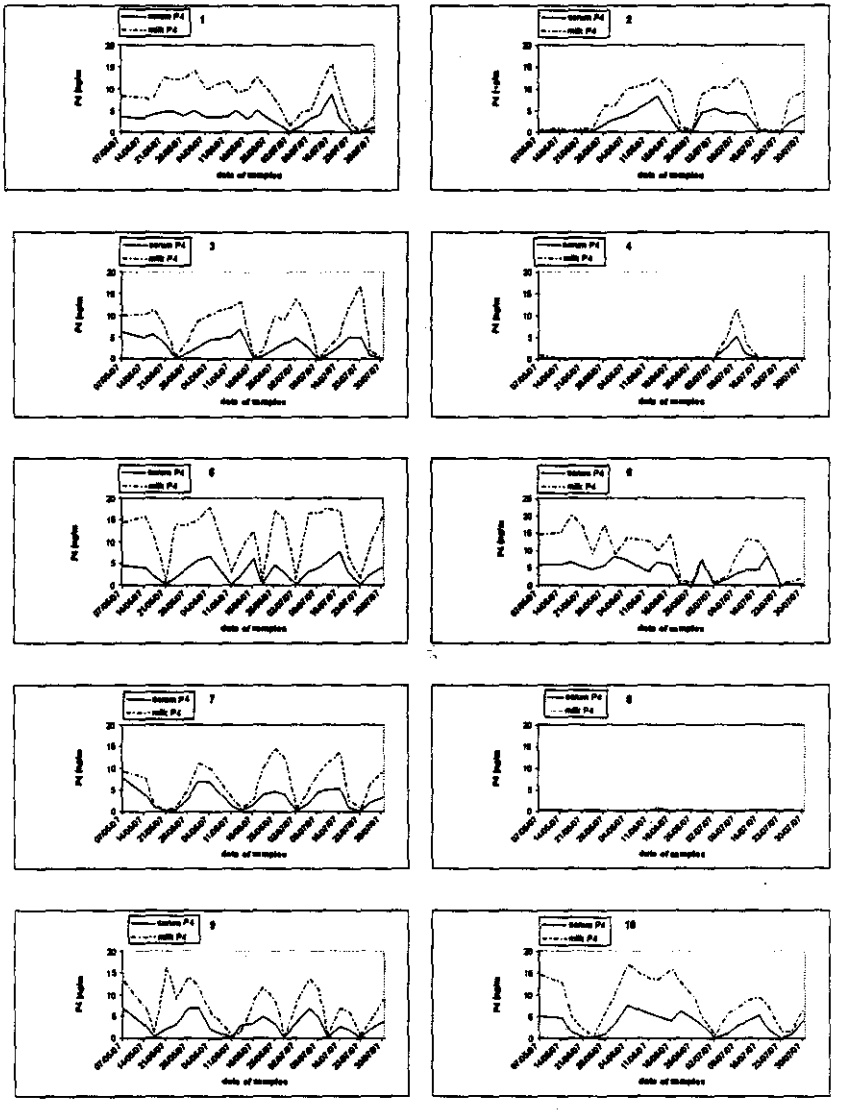
In non-pregnant buffalo, the highly significant ($p<0.001$) correlation coefficient between P_4 levels in milk and serum ($r=0.87$) suggests that P_4 determination in milk could be useful indicator for diagnosing ovarian dysfunction in buffalo especially if assessed in repeated consecutive samples.

It was also shown that all pregnant buffalo had P_4 levels > 3.0 ng/ml in milk. In addition, all buffalo cows with milk P_4 levels of < 3.0 ng/ml were non-pregnant. That is to conclude that dairy buffalo with < 3.0 ng/ml P_4 level in milk could be diagnosed non-pregnant with high degree of accuracy. Otherwise, the use of P_4 level of > 3.0 ng/ml in milk could only be suggestive for the occurrence of pregnancy. The possibly encountered cases of ovarian luteal cysts or persistent CLs could interrupt the accuracy of diagnosis.

In conclusion, the P_4 assay in fat-free buffalo milk could be used as an efficient mean for diagnosing non-pregnant buffalo with high degree of accuracy. Otherwise, it is tentatively suggestive for diagnosing pregnant

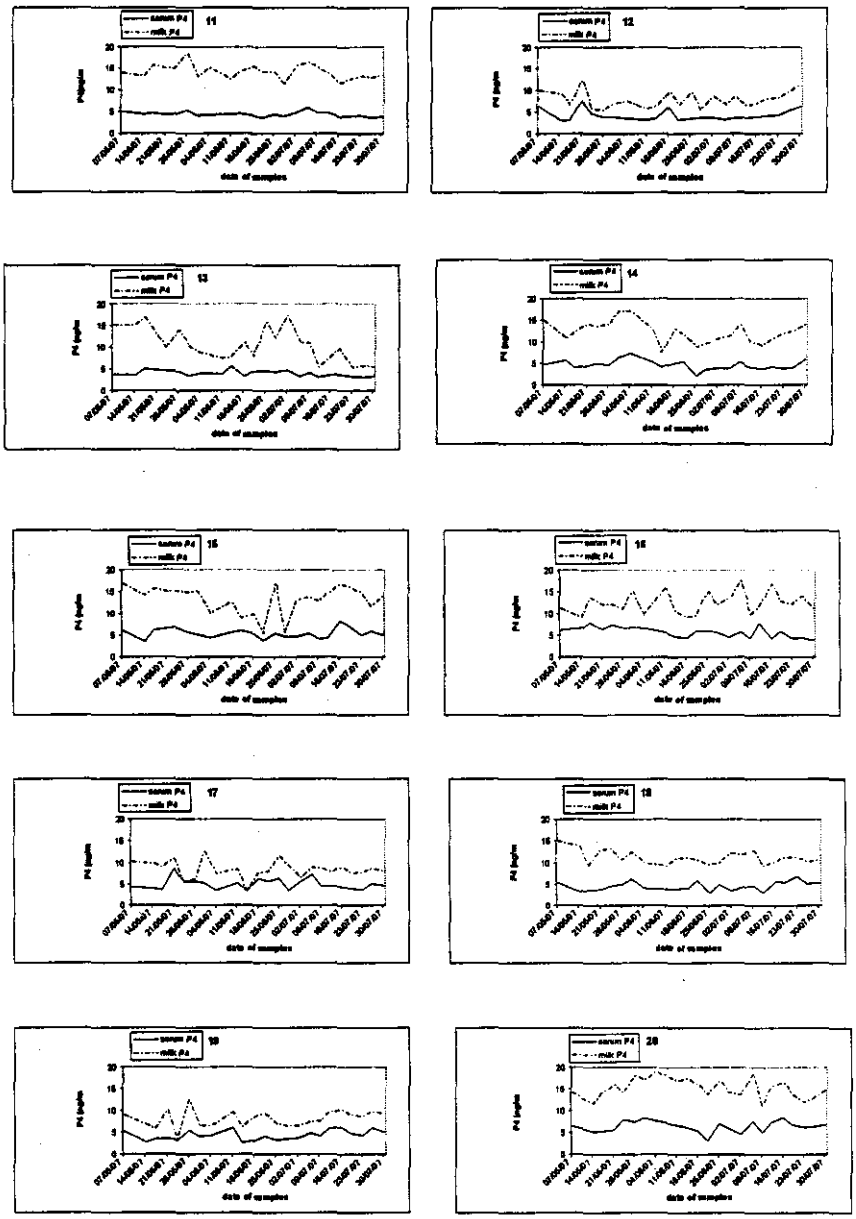
buffalo. This assay could also be suggested as an easy and useful mean for monitoring ovulation, ovarian dysfunction, embryonic mortality and pregnancy in buffalo, especially under rural conditions.

Plate 1



Milk and serum P_4 profiles in individual non-pregnant buffalo cows.

Plate 2



Milk and serum P_4 profiles in individual pregnant buffalo cows.

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إستخدام بروجسترون اللبن لمتابعة التبويض، الدورات المبيضية، والحمل فى الجاموس

خالد توفيق عثمان*، حسين أحمد الرجلاتى*، أحمد عصام فكرى، حسن مصطفى فرغلى ** و حسن بيومى أبو العلا***

* معهد بحوث الإنتاج الحيوانى، مركز البحوث الزراعية، وزارة الزراعة.

** مركز الطاقة النووية، هيئة الطاقة الذرية.

أجرى هذا البحث بغرض دراسة العلاقة بين مستويات هرمون البروجسترون فى سيرم دم ولبن الجاموس منزوع الدهن. كذلك فقد تناول البحث تقييم كفائة إستخدام مستوى بروجسترون اللبن كمؤشر لمتابعة الأداء التناسلى للجاموس. وقد إستخدم فى الدراسة عدد ٢٠ جاموسة مابين الموسم الثانى والسابع (عدد ١٠ حوامل، و١٠ غير حامل). كذلك فقد تم تقدير مستويات هرمون البروجسترون فى عدد ٤٦٠ عينة سيرم دم، و ٤٦٠ عينة لبن منزوع الدهن- تم التحصل عليها من الحيوانات التجريبية. وقد تم حساب معاملات الإرتباط مابين تركيزى الهرمون فى السيرم واللبن لكل من الجاموس الحامل وغير الحامل. كذلك فقد تم توقيع خرائط بيانية للتغيرات الحادثة فى مستويات الهرمون خلال المراحل الفسيولوجية المختلفة لكل حيوان على حدة. هذا، وقد إستخدمت تقديرات البروجسترون بكل من اللبن والسيرم فى توصيف أنماط الدورات المبيضية، تشخيص حالات الخلل الوظيفى للمبيض، وكذلك فقد الأجنة بالحيوانات التجريبية.

أظهرت النتائج أن جميع تقديرات الهرمون باللبن كانت أعلى من نظيراتها بسيرم الدم (P<0.01). وقد تراوح تركيز الهرمون فى الحيوانات الحامل بين ٢,٠- ٨,٥ نانوجرام/مل فى السيرم، و ٣,١- ١٨,٦ نانوجرام/مل فى اللبن، بينما تراوحت التركيزات المقابلة للهرمون فى الحيوانات غير الحامل بين ٠,١ - ٨,٥ و ٠,١ - ١٩,٩ نانوجرام/مل فى كل من السيرم واللبن على الترتيب. هذا، وقد إختلفت تركيزات الهرمون معنويا (P<0.01) بين كل من المجموعتين التجريبيتين- وكذلك داخل كل مجموعة، وكان متوسط تركيز الهرمون فى لبن الجاموس الحامل (١١,٣ ± ٠,٢ نانوجرام/مل) أعلى ٢,٣ مرة عن تركيزه فى السيرم (٤,٩±٠,١ نانوجرام/مل) بينما كان متوسط تركيزه فى لبن الجاموس غير الحامل (٦,٨±٠,٥ نانوجرام/مل) أعلى ٢,٥ مرة عن تركيزه فى السيرم (٢,٧±٠,٢ نانوجرام/مل).هذا، وقد كانت معاملات الإرتباط بين تركيزات الهرمون فى كل من اللبن والسيرم عالية المعنوية (P<0.001) لكل من الجاموس الحامل (r=0.50) والجاموس غيرالحامل (r=0.87).

كان المتوسط العام لطول دورة التبويض هو ٢٠,٢±١,٥ يوما، وقد شكلت الدورات الطبيعية (١٨-٢٤ يوما) نسبة ٥٠% من اجمالى عدد الدورات، بينما كانت نسب حدوث الدورات القصيرة (≤ ١٧يوم) والطويلة (≥ ٢٥ يوما) هى ٣١.٨، ٢ .١٨% - على الترتيب. هذا، وقد إنحصرت جميع التبويضات فى هذه الدراسة عند مستوى بروجسترون مابين ٠,١ - ٢,٩ نانوجرام/مل فى اللبن ومستوى ٠,١ - ٠,٤ نانوجرام/مل فى السيرم. كذلك، فقد تراوح أقصى مستوى للهرمون أثناء فترة منتصف دورة التبويض مابين ٦,٧ - ١٧,٨ نانوجرام/مل فى اللبن و ٢,٦ - ٨,٥ نانوجرام/مل فى السيرم. لذا فإنه يمكن القول أن الإنخفاض المفاجئ للهرمون باللبن إلى مستويات تقل عن ٢,٩ نانوجرام/مل بعد فترة إرتفاع (≥ ٢,٩ نانوجرام/مل-على مدارعينتين أو أكثر) يمكن أن يستدل منه على حدوث التبويض، كما أنه قد يكون مؤكدا لحدوث الشياع. وقد أكدت الدراسة على ضرورة تقدير بروجسترون اللبن فى عدة عينات متتالية- تحقيقا لرؤية أعمق ودقة أعلى للحكم على حدوث التبويض، ذلك حيث أن تأكيد التبويض على أساس عينة واحدة قد يؤدى إلى نتائج مضللة وخصوصا فى الحيوانات غير منتظمة الدورة التى تعانى من خمول مبايض أوتكيسات مبيضية حويصلية.

أوضحت نتائج الدراسة أن جميع تركيزات البروجسترون فى لبن الجاموس الحامل كانت > ٣,٠ نانوجرام/مل، كذلك فقد كانت جميع تركيزات البروجسترون فى لبن الجاموس غير الحامل < ٣,٠ نانوجرام/مل. لذا، فقد أمكن إستنتاج أن الجاموس الذى يقل فيه تركيزبروجسترون اللبن عن ٣,٠ نانوجرام/مل يمكن تشخيصه "غير حامل" بدرجة عالية من الدقة، بينما الجاموس الذى يزيد فيه تركيز بروجسترون اللبن عن ٣,٠ نانوجرام/مل فإنه لا يجب أن يشخص "حاملا " بدرجة عالية من التأكيد (نظرا لإحتمالية تعارض دقة الحكم مع وجود تركيبات مبيضية مفرزة للبروجسترون مع غياب الحمل).

وقد خلصت الدراسة إلى إمكانية إستخدام تقديرات بروجسترون لبن الجاموس "منزوع الدهن" كوسيلة سهلة يمكن من خلالها متابعة الأداء التناسلى للجاموس- خاصة تحت الظروف الحقلية. هذا، وقد أوضحت الدراسة كفائة إستخدام تقدير بروجسترون اللبن لتأكيد التبويض، تشخيص الخلل الوظيفى للمبيض، ومتابعة الحمل فى الجاموس. كذلك فقد أكدت النتائج إرتفاع كفائة التقديرعند تشخيص الجاموس غير الحامل، وبدرجة أقل من الكفائة عند تشخيص الجاموس الحامل. هذا، وقد إقترحت الدراسة ملائمة إستخدام تقديرات بروجسترون اللبن كوسيلة ميسورة لمتابعة الأداء التناسلى للجاموس تحت ظروف الريف المصرى.

قام بتحكيم البحث

أ. د/ مصطفي عبد الحليم الحرايري — كلية الزراعة – جامعة المنصورة

أ. د/ فكري السيد القربي — مركز البحوث الزراعية