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SERUM AND MILK PROGESTERONE MONITORING AND ITS RELATION TO OVARIAN CONDITIONS IN EGYPTIAN BUFFALOES

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A B S T R A C T

To investigate the correlation between ovarian structures and progesterone (P4) concentrations in Egyptian buffaloes (n=80), blood and milk samples were in association with gynecological examination. There was a positive correlation (r=0.38, P<0.005) between serum and milk P4 levels and the presence of CL resulted in an elevation of P4 (P<0.0001) as compared with smooth inactive ovary. The highest P4 levels were recorded with persistent CL, but the comparatively low levels were estimated during pregnancy. There was a substantial decrease in P4 with progression of pregnancy, but higher levels (P<0.05) were recorded at the 2nd month. P4 levels in serum and milk were close at the 1st and 3rd months, while a gap between both levels was significant at the 2nd, 3rd and 5th months of pregnancy. In conclusion, P4 levels is an indicator to the reproductive status associated with presence of luteal tissue especially persistent CL in Egyptian buffaloes.

KEY WORDS: Buffalo, Milk, Ovary, Progesterone, Serum.

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1. I N T R O D U C T I O N

Concurrent changes in plasma levels of estrogen and progesterone are most likely to be responsible for the manifestation of estrus behavior and subsequent ovulation [1]. In general, progesterone concentration is usually low (~ 0.1 ng /ml) in peripheral blood circulation of buffaloes at estrus and doesn't rise until day 3-5 after estrus [1]. In Egyptian buffaloes, Shafie et al. [10] found that progesterone increased gradually from non-detectable values (< 0.1 ng/ml) at the heat period to reach the maximum concentration (5.5 ng/ml) on day 17 of the cycle before declining rapidly to level of < 0.1 ng/ml during the rest of the cycle. Milk samples can be taken for progesterone assay as an indicator of the ovarian activity and pregnancy reflecting the activity of CL [7]. Souza et al. [12] found that maximum concentration of milk progesterone in crossbred buffaloes (Mediterranean × Murrah) at the peak of the cycle varied from 1.31 - 3.35 ng/ml and at estrus was 0.5 ng/ml. The aim of current was to investigate the correlation between serum and milk progesterone levels during different ovarian conditions in the Egyptian buffaloes.

2. MATERIAL AND METHODS

2.1. *Animals and experimental design*:
The present study was conducted on a total number of 80 buffaloes 3 - 9 years old at 1-6 lactations, diagnosed as free of reproductive disorders belonged to the Experimental Farm of Animal Production Department, Faculty of Agriculture, Minufiya University during the period from November 2009 to March 2012. All

animals were naturally bred at the appearance of first heat following calving and non-return buffaloes after 3 weeks after mating were left to be palpated rectally at about 60 days later for pregnancy diagnosis.

2.2. *Blood and milk sampling and hormonal assay*:

Milk (10 ml) and blood (6 ml) samples were collected prior to each rectal palpation and ultrasonography examination which has been performed using a 6/8 MHz linear array trans-rectal transducer (Pie Medical 240 Vet®, Pie Medical Equipment B.V., Maastricht, Holland)) according to the methods described by Kähn [6]. Sera were harvested after centrifugation (3000 rpm for 20 min.) within 2-4 hours of collection and stored at -20°C. Morning milk samples (8-10 ml) were collected after removing the fat layer by means of centrifugation (3000 rpm), 0.2 mg of potassium dichromate was added to milk sample as a preservative and were stored at -20 °C until assayed for progesterone. The progesterone hormone was estimated in collected samples (serum and milk) by using the coated tube progesterone ELIZA (solid phase Enzyme-linked Immunosorbent Assay) Kits (DRG International, Inc, Germany) based on the principle of competitive binding according to manufacturer manual. All assays were carried out by ELIZA Reader: Stat Fax 2100 (Awareness Technology, Inc., USA).

2.3. *Statistical analysis*:

Data were collected, tabulated and statistically analyzed to detect the inter-relationship between various estimated serum and milk hormonal correlations by student *t*-test and one way ANOVA using SPSS program (Ver. 16).

3. RESULTS AND DISCUSSION

Buffaloes had cyclic CL on their ovary in this study showed a highly significant ($P<0.0001$) increase in serum and milk P4 level as compared with those had smooth inactive ovary (table, 1). The highest P4 level (3.31±0.27 ng/ml and 4.40±0.16 ng/ml in serum and milk, respectively) was recorded in presence of persistent luteal tissue. In contrary, the overall mean of P4 level revealed was comparatively low in presence of pregnancy CL (0.97±0.10 ng/ml and 1.95±0.13 ng/ml in serum and milk, respectively).

Earlier studies showed that P4 rise to maximum concentration (1.6–3.6 ng/ml) on day 13–15 of the estrous cycle [13] before declining rapidly to level of < 0.1 ng/ml during the rest of the cycle [10]. In heifers, Berardinelli et al. [2] showed that the plasma P4 increased from 0.7 to 2.1 ng/ml with the existence of luteal tissue.

Monitoring P4 level in buffalo in the current work showed a substantial decrease with progression of pregnancy during the monitored period (five months).

Table 1 Effect of ovarian status on serum and milk progesterone

Ovarain status		Progesterone level			
	n	Serum	Milk	r value	P value
1-Day of calving	20	2.57 ± 0.34^{ab}	2.60 ± 0.27^{b}	-0.08	ns
2- Smooth ovary	3	0.60 ± 0.15^{d}	0.42 ± 0.11^{d}	0.11	ns
3- Large follicle	5	1.81 ± 0.60^{bc}	2.97 ± 0.82^{b}	0.63	ns
3-Cyclic CL	16	3.038 ± 0.34^{a}	3.85 ± 0.37^{ab}	0.26	0.06
4-Persistent CL	24	3.31 ± 0.27^{a}	4.40 ± 0.16^{a}	0.42	<0.05
5-Pregnancy CL	12	0.97 ± 0.10^{c}	1.95 ± 0.13^{c}	0.34	0.09

Values (Mean ±SEM) within the same column with different superscripts were statistically significant ($P<0.05$). Buffalo (n=12) samples during pregnancy (5 months) were pooled to calculate the overall mean of progesterone level during pregnancy.

Concurrently El-Sheikh et al. [3] declared that the progesterone content and concentration in the CL increased consistently and reached its peak in the second period of pregnancy, then decreased gradually until the fourth period and this reflected the histomorphological changes characterized by the presence of numerous active cells in the corpus luteum in the first two periods of gestation and the occurrence of retrogressive changes in the CL in third period of gestation.

Progesterone level in serum was significantly ($P<0.05$) higher at the first and second months samples compare to that recorded on the fifth month of pregnancy. In the meantime, progesterone level in samples collected at the second and third was significantly ($P<0.05$) higher than that recorded on the fifth month of pregnancy (fig. 1). This finding might be attributed to difference in fat content as the percentage of fat and the progesterone concentration in the milk were correlated [11]. Kamonpatana et al. [8] showed that milk progesterone in fat and whole milk indicated that the dairy breeds had higher progesterone levels than non-dairy breeds.

Statistical analysis in the current study revealed a close highly significant ($P<0.005$) positive correlation ($r=0.38$) between serum and milk progesterone (P4) levels in the examined samples representative various ovarian conditions (fig. 2). It has been well established that monitoring the P4 level during the postpartum period as a good indicator for changes in ovarian activity and the function of corpus luteum (CL) [4]. O'Connor [9] revealed that milk progesterone analysis isn't used to verify suspicious heats and the cow was observed in heat only, but also can be used for diagnosis pregnancy. Besides, it can be used to speculate abnormally long inter-estrous interval, evaluate accuracy of heat detection or identify errors in estrus detection, identify open cows, monitor postpartum ovarian status, differentiate types of ovarian cysts, evaluate response to various hormonal treatments.

Presence of persistent CL on the ovary was associated with a significant ($P<0.05$) positive correlation ($r=0.42$) between serum and milk P4, while in cases with cyclic and pregnancy CL the hormonal level tended to be significantly correlated ($r=0.26$, $P=0.06$ and 0.34, $P=0.09$, respectively). In cows, progesterone level, in case of persistent CL was estimated to be in milk ≥ 3 ng/ml (Garmo et al., 2009).

Monitoring P4 levels in serum and milk in the current study revealed that the milk P4 was higher than its representative level in serum throughout the study. Both hormone levels were close during the first month of pregnancy (2.36±0.39 ng/ml and 2.65±0.49 ng/ml, respectively). The dissimilarity between both hormonal levels was significantly evident on the 2nd and 3rd months before return to be close on the 4th month and became significantly differed thereafter at the 5th month of pregnancy (fig. 1). In Murrah buffaloes, progesterone concentration in whole milk was four to five times higher than its level in the blood circulation [11].

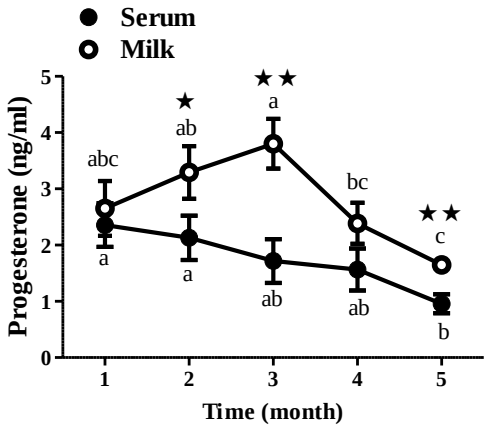

Fig. 1 Progesterone hormone levels in Egyptian buffaloes during pregnancy. Values presented (mean± S.E., n=12) within the same type (serum (●) or milk (○)) with different superscript letter were significantly different. ★, ★★ indicated significant variances between measured hormonal levels at the same time point $P<0.05$ and $P<0.01$, respectively.

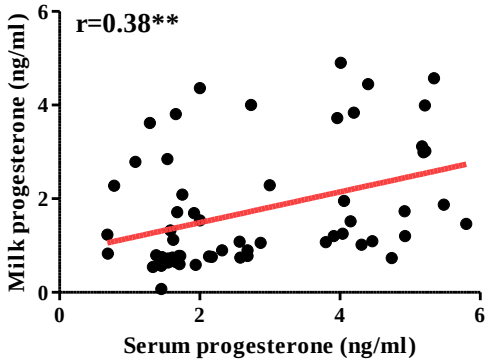

Fig. 2 Correlation between serum and milk progesterone concentrations measured in different cases of ovarian activity. Red line indicated the regression line. ** indicated (P<0.005).

From this study we can conclude that serum and milk progesterone levels were more significant and positively correlated alongside with the persistence of CL than during estrous cycle and pregnancy. The substantial decline in progesterone with advancement of pregnancy might be a unique phenomenon to buffaloes probably due to the retrogressive changes in luteal tissue.

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قياس هرمون البروجسترون فى الدم واللبن وعلاقته بحالة المبيض في الجاموس المصري

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الملخص العربى

هدفت هذه الدراسة الى التحقق من العلاقة بين الاجسام المبيضية ومستوى هرمون البروجسترون في الدم واللبن الجاموس في المصري. تم تجميع عينات من الدم واللبن بالتزامن مع فحص التناسلى باستخدام الموجات الفوق صوتية من ثمانون حالة. اظهرت نتائج الدراسة وجود ارتباط إيجابي و معنوى بين مستويات هرمون البروجسترون فى الدم و اللبن، و وجود جسم اصفر على المبيض و المصحوب بارتفاع مستويات هذا الهرمون. تم تسجيل أعلى مستويات هرمون البروجسترون مع وجود جسم اصفر مستمر(حالة مرضية) على المبيض، كان المستوى العام لمتوسط هرمون البروجسترون خلال فترة العشر منخفضاً على الرغم تسجيل أعلى مستويات للهرمون في الشهر الثانى. اظهر هرمون البروجسترون وجود ارتباط وثيق بين نسبته فى الدم و اللبن خلال الثلاث أشهر الاولى من العشر، بينما كانت توجد فجوة بين المستويين في الشهر الثالث و الخامس من العشر. من هذه الدراسة نخلص الى ان مستويات هرمون البروجسترون فى الدم واللبن تعتبر مؤشراً للحالة التناسلية للجاموس و بالاخص المرتبطة بوجود جسم أصفر مستمر (حالة مرضية) في الجاموس المصري.

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