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MILK PROGESTERONE AS AN INDICATOR OF REPRODUCTIVE STATUS IN EGYPTIAN BUFFALO COWS

A.F. Nebar[1], H. Abdel Rahman[1], A.A. Habeeb[2] and R.S. Mourad[1]
1- Animal production Department, Faculty of Agricultural, Menofiya University
2-Biological Application Departement, Atomic Energy Authority, Inshas.

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***ABSTRACT:** A total 179 milk samples were collected from 27 buffaloes which were classified into four groups according to their reproductive status to study the possibility of using concentration of milk progesterone (MP) as an indictor of reproductive status in Egyptian buffaloes. Group 1 included 7 buffaloes represented the postpartum anestrous and uterine involution, milk samples in this group were collected from day 19 to day 47 after calving. Group 2 involved 7 buffaloes being inseminated at the first postpartum estrous and did not express estrous behavior after insemination but rectal palpation (60 days post breeding) revealed that none of them were pregnant. Group 3 included 7 buffaloes to illustrate the changes in MP on days 3, 15, 18 and 21 after fertile service. Group 4 included 6 buffaloes represented the changes in MP level after fertile service on days 0, 2, 6, 10, 14, 18, 20 22, 25 and 42.*

Results revealed that the overall average of MP concentration during uterine involution period was 0.91±0.18, ranging from 0 to 8.25 ng/ml. Results indicated that fertile insemination (G3) was accompanied with higher MP concentration on days 3, 15, 18 and 21 post breeding than that with the infertile services of G2 (1.2, 3.88, 4.42 and 4.37 vs. 0.68, 2.0, 2.42 and 2.49 ng/ml, respectively). Infertile services in G2 were accompanied with some ovarian disorders (42.9% persistent CL, 28.6% smooth ovaries, 14.3% cystic ovary and 14.3 normal ovarian structure). High MP levels obtained in G2 especially on day 21 suggested false pregnancy diagnosis in about 57.1% of these animals. The average MP level in the first postpartum fertile service significantly differed ($p<0.01$) from 0.16 on day 0, increased progressively with the advance of the post service period, reaching its peak (15.37 ng/ml) on day 14 and remained thereafter over 6.0 ng/ml up to day 42 after service. Clear evidences suggested that the accuracy of pregnancy diagnosis obtained from the present study through the MP was about 86.0%.Thus concentration of MP could be used as an indicator of the reproductive status either for pregnancy diagnosis with accuracy of 86.0% or identifying the ovarian activities. It is more economic to determine MP concentration on day 24 after service.

***Key words:** Buffaloes, Milk Progesterone, Pregnancy, Reproduction*

INTRODUCTION

Undoubtedly, buffaloes have a great impact on the national agriculture income of Egypt. They produce about 65% and 45% of the total national milk yield and meat production, respectively. However, buffaloes are characterized by poor reproductive performance as compared to cattle (Gordon, 1996). In this respect, calving interval in Egyptian buffaloes varied from 500 to 605 days (Eid, 1988; Mokhless *et al*. 1995 and Mahdy *et al.* 2001) resulted in substantial economic losses. Early detection of pregnancy is imperative for reduction the open days, which result in minimization the calving interval. Blood and milk progesterone concentration could be used for early pregnancy diagnosis as well as for detection of silent and anovulatory heat periods in buffaloes (Kamonpatana *et al.* 1981; Sharma *et al.* 1990; Raggi *et al.* 1999 and Qureshi *et al*. 2000). The objectives of this study aimed to declare the possibility of using milk progesterone concentration as an indicator for normal cyclicity, pregnancy diagnosis and reproductive disorders in the Egyptian buffaloes.

MATERIALS AND METHODS

The present study was carried out at the Experimental Farm of Animal Production Department, Faculty of Agriculture, Minufiya University. Twenty seven buffalo cows, aged 3 - 9 years and weighed from 345 to 570 kg and varied between first and fourth parity were used in this study. All animals were healthy, free from internal and external parasites or any obvious pathogenic diseases, and housed in well ventilated and naturally lightened close system. The feeding allowances were calculated according to live body weight and average daily milk yield of buffalo in each group as described by Ghoneim, (1964). Feed mixture containing 60% yellow corn, 15% wheat bran, 23% soybean meal, 1% sodium chloride and 1% mineral mixtures, green clover (*Trifolium alexandrium*) and rice straw were offered *ad-libitum* from November to June. During the remainder period of the year the green clover was replaced with clover hay. All buffalo cows were machine milked twice daily. The non lactating female buffaloes also received their concentrate allowance at the same time likewise the lactating ones. The experimental animals were classified into 4 groups according to their reproductive status as follows:

Group 1: **Seven buffaloes represented the postpartum uterine involution and anestrus. Milk samples were collected from day 19 to day 47 after calving with 3-4 days apart.**

Group 2: **Seven buffaloes, which were naturally inseminated at the first postpartum estrus and did not show any estrus behavior after being naturally inseminated. Milk samples were collected on days 3, 15, 18 and 21 after the service. All animals were rectally palpated after 60 days of service and none of them were pregnant. Rectal palpation revealed also that three of them had**

persistent corpus luteum (CL), two with smooth ovaries, one with cystic ovary and the remaining one had normal ovarian structure.

Group 3: Seven buffaloes were selected to illustrate the changes in the milk progesterone (MP) on days 3, 15, 18 and 21 after the first postpartum fertile service.

Group 4: Six buffaloes represented the changes in MP after the first postpartum fertile service. Milk samples were collected on days 0, 2, 6, 10, 14, 18, 20, 22, 25 and 42 after insemination (day 0 = insemination day).

Milk samples were collected from the afternoon milking, after thoroughly mixing of milk yield. In this respect, about 8 – 10 ml of milk was taken into a clean dry vials containing 0.2 mg of potassium dichromat as a preserving agent. After mixing, the samples were stored frozen at -20°C until progesterone assay.

Milk progesterone was estimated by the radioimmunoassay (RIA) technique using the coated tube kits according to Haynes *et al*. (1980) and Blight and White (1983). Obtained data were statistically analyzed according to SPSS (1997) program.

RESULTS AND DISCUSSION

Milk progesterone concentration during postpartum anestrus and uterine involution period:

None of the buffaloes in this group expressed estrual behavior during the period from day 19 up to 47 after calving. In other words, during this period the animals were still in postpartum anestrus. Table 1 shows that the overall average of MP concentration during uterine involution period was 0.91 ± 0.18 ng/ml, ranging from 0 to 8.25 ng/ml. A remarkable fluctuation in MP during this period was detected being 0.86 ± 0.31 ng/ml on day 19, increased to 1.36 ± 1.04 ng/ml on day 22, then it progressively decreased reaching a minimum average (0.30 ± 0.17 ng/ml) on day 36 and increased once again to 1.85 ± 0.93 ng/ml on day 43 post partum. Statistical differences among periods were not significant. Average MP was below 0.60 ng/ml in 5 out of 7 buffaloes (71.4%), meanwhile it was 2.35 ng/ml in the remains 2 buffaloes (28.6%), as indicated in Table, 1. None of these buffaloes show any symptoms of estrual behavior during 47 days after calving. In the same time, they also did not show two successive MP elevations, proving true anestrus. It is of interest to note that one of 7 buffaloes (No.6) showed considerable MP elevation from 2.06 ng/ml on day 19 to 7.54 (exhibiting progesterone spike), 3.03, 2.48 ng/ml on days 22, 26, 29 then it started to decrease to 1.75 and 0.23 ng/ml on days 32 and 36 postpartum. This finding suggested a possible quite ovulation (silent heat).

However, another buffalo cow (No.7) exhibited progesterone spike between days 39-47 postpartum, suggesting a possible quite ovulation (silent heat) started on day 36 postpartum.

Table (1): Individual milk progesterone concentration (ng/ml) in 7 buffaloes during postpartum anestrous period (Group 1).

Days Post-Partum	Buffalo number							Aver. ± SE
	1	2	3	4	5	6	7	
19	0.28	0.42	0.48	0.00	0.79	2.06	1.97	0.86 ± 0.31
22	0.36	0.71	0.06	0.00	0.17	7.54	0.69	1.36 ± 1.04
26	0.55	0.32	0.00	0.00	0.00	3.03	2.31	0.89 ± 0.47
29	0.004	0.00	0.33	1.59	0.33	2.48	0.05	0.68 ± 0.37
32	0.07	0.00	0.08	0.77	0.02	1.75	0.57	0.47 ± 0.24
36	0.07	0.00	0.39	0.17	1.25	0.23	0.00	0.30 ± 0.17
39	0.00	0.00	0.00	0.65	0.00	1.91	4.41	1.00 ± 0.63
43	0.65	0.69	6.26	0.91	0.003	1.17	8.25	1.85 ± 0.93
47	0.14	0.86	0.31	0.00	0.43	0.97	2.92	0.80±0.38
Average ±SE	0.24^{b} ±0.08	0.33^{b} ±0.12	0.54^{a} ±0.34	0.45^{a} ±0.19	0.55^{a} ±0.15	2.35^{a} ±0.71	2.35^{a} ±0.88	0.91±0.18

Means followed by different letters are significantly differed (P <0.01)

Data available in the literature illustrate that the basal level of milk progesterone in buffaloes varied from 0.05 ng/ml to a peak values of 8.58-20.2 ng/ml during the postestrus period (kamnonpatana *et al*., 1983). These findings are in quite agreement with the present study. Also, Perera *et al*. (1984) stated that the first marked postpartum progesterone elevation (<0.7 ng/ml) was preceded by a minor elevation for a short duration (0.3-0.6 ng/ml). However, Beg and Totey (1999) indicated that the plasma progesterone during the postpartum anestrous period in buffaloes was 0.20-0.30 ng/ml. The observed low MP level during this period is in agreement with that obtained by El-Moghazy (2003), who found that MP concentration was sustained at a low level of 0.32 ng/ml. Such finding may be attributed to the high level of $PGF_2\alpha$, which leads to Cl regression of pregnancy (Barkawi, 1984).

Milk progesterone profile after the first postpartum infertile service:

Data in Table 2 show changes in MP concentration of 7 buffaloes in group 2 on days 3, 15, 18 and 21 after the first postpartum infertile service. Although the animals did not return to estrus after service, the rectal palpation revealed that they did not conceive.

Table (2): Individual changes of milk progesterone concentration (ng/ml) and ovarian examination of 7 buffaloes after the first postpartum infertile service (Group 2).

Buffalo number	Day after first postpartum infertile service				Ovarian examination
	3	15	18	21	
1	0.74	2.39	1.42	2.13	Normal structure
2	0.00	0.00	0.56	3.23	Persistent CL
3	0.68	1.19	0.31	0.91	Cystic ovary
4	0.37	1.38	1.25	0.79	Smooth ovary
5	0.03	5.15	8.21	6.22	Persistent CL
6	2.87	2.32	3.75	3.95	Persistent CL
7	0.00	1.56	1.46	0.17	Smooth Ovary
Average± SE	0.68 ±0.38	2.00 ±0.61	2.42 ±1.05	2.49 ± 0.81	

Average MP on day 3 after the first postpartum infertile service (post-estrus) was 0.68±0.38 ng/ml, increased to 2.00±0.61 and 2.42±1.05 ng/ml on days 15 and 18 of the service and 2.49±0.81 ng/ml on day 21 with no significant differences. It clearly appears that 3 out of 7 buffaloes (No.3, 4, 7) in this group showed lower MP concentration on day 21 (below 1.0 ng/ml), suggesting the end of luteal phase and starting a new estrus cycle. Where, cows require progesterone to maintain pregnancy and cows with low concentration of progesterone are extremely unlikely to be pregnant. However, the absence of returning to estrus conflicted this suggestion. On the meantime, the elevation of MP concentration on day 21 in other 4 buffaloes and non-return to next estrus after the first postpartum service suggested the possibility of being pregnant. Rectal palpation after 60 days revealed the opposite. This means that, in this case, using the MP concentration on day 21 for diagnosis pregnancy in buffaloes is of lower validity. Elevated milk progesterone on days 20 to 24 after insemination only indicates the presence of corpus luteum,

which is usually but not invariably, associated with a conceptus. According to the obtained data, 42.8% of the buffaloes were non pregnant due to the lower MP (from 0.17 to 0.91ng/ml) on day 21 post service, versus 57.2% of the buffaloes gave false pregnancy diagnosis due to incidence of persistent CL and/or possible early embryonic mortality. Buffaloes in this group exhibited ovarian disorders, where 42.8% had persistent CL, 28.6% had smooth ovaries, 14.3% had cystic ovary and 14.3% had normal ovarian structure.

In this respect, Kamonpatana *et al*. (1983) and Kaker *et al*. (1993) mentioned that progesterone concentration displays cyclic changes from phase to phase of the estrus cycle. Rao and Pandey (1982) found that plasma progesterone concentration on day 4 of the estrus was 0.96 ng/ml in Murrah buffaloes during warm or hot season being slightly greater than 0.68 ng/ml for MP concentration obtained on day 3 of the cycle (Table, 2).

During estrus and pro-estrus phases, Kamonpatana *et al*. (1976 and 1978) found the progesterone level was less than 1.0 ng/ml in Swamp buffaloes, it increased to be 1.8 ng/ml after 1-2 days post-estrus and reached a peak (3.9 ng/ml) on day 16 of the cycle. Thereafter, it declined rapidly, 4-7 days before the next estrus phase. Also, Vale *et al*. (1990) reported that MP concentration at estrus was 0.33 - 0.48 ng/ml, increased to 1.3 – 1.8 ng/ml on days 3-5 (post estrus), then mounted to 3.1 – 4.8 ng/ml on days 15 – 17 and fell to 2.3 – 2.8 ng/ml on days 20 – 21 of the cycle. These findings show some disagreement with the pattern of change in MP obtained in the present study, especially regarding the decline in MP level after day 17 of the cycle detected by Vale *et al*. (1990). However, their obtained value was quite close to that given in Table, 2. The disagreement in the pattern of the cycle changes in MP concentration could be explained on the basis of the ovarian disorders detected by rectal palpation in the present study. Vandewile *et al*. (1979) demonstrated that MP over 5.0 ng/ml could be considered as indicator of luteal activity. This is clearly appeared in buffaloes with persistent CL in the present study where MP concentration was 5.15, 8.20 and 6.22 ng/ml on days 15, 18 and 21 of the cycle. In one buffalo of those having persistent CL (No.2), although she (No.2) had high MP level (3.23 ng/ml) on day 21, it preceded by lower concentration of MP (<0.56 ng/ml) on day 18. This may suggest that the start of the persistent CL was delayed to the end of the cycle and continued up to the rectal palpation ovarian examination on day 60 after service.

Batra *et al*. (1979) found MP level in non pregnant Murrah buffaloes to be 2.7 ng/ml on day 3 post-estrus and the peak values were 18.5 and 15.3 ng/ml on days 15 and 18 of the cycle, respectively. These values (18.5 and 15.3 ng/ml) were comparatively greater than that obtained in the present study, which may be due to the procedure of milk sampling.

Milk progesterone profile after the first postpartum fertile service:

Table, 3 shows the individual cyclic changes of MP in 7 buffalo cows during the first 21 days after fertile service. The tabulated data indicated that the average MP concentration on day 3 (postestrus) was 1.20 ng/ml and it progressively ($p<0.01$) increased to be 3.88, 4.42 and 4.37 ng/ml on days 15, 18 and 21, respectively.

Table (3): Individual changes in milk progesterone concentration after the first postpartum fertile service in buffaloes (Group 3).

Buffalo number	Days after fertile service			
	3	15	18	21
1	1.04	4.93	4.50	4.31
2	2.17	3.84	3.85	4.37
3	1.32	4.24	6.57	7.57
4	1.13	1.14	2.76	1.80
5	2.35	2.91	3.17	2.07
6	0.39	7.03	6.14	7.11
7	0.00	3.08	3.97	3.36
Aver. ± SE	1.20 ± 0.32^{b}	3.88 ± 0.69^{a}	4.42 ± 0.54^{a}	4.37 ± 0.85^{a}

Means followed by different letters are significantly differed ($P<0.01$)

Data in Table, 4 show that the average MP concentration proved significant ($p<0.01$) progressive increase with the advance of the period up to 14 days after the first postpartum fertile service, when the MP level reached its maximum values (15.37 ng/ml). Thereafter, MP level showed marked decrease up to 42 days after service, but still above 5.0 ng/ml. Rectal palpation after about 2 months of the natural insemination day revealed that 5 out of 6 animals were pregnant (83.3%) and only one was non gravid (16.7%), but it inseminated and conceived after 16 days of the rectal palpation. The MP level in Table, 4 of this animal (No.4) and the absence of estrous behavior symptoms as well as the long period between the two successive natural insemination and exhibition of estrus and conception (after 76 days) strongly suggested incidence of early embryonic mortality. This was verified also by rectal palpation, which proved slight difference in size of the two uterine horns of this buffalo, the non gravid uterus with existence of regressed CL, all of these phenomena beside the high concentration of MP up to day 42 after the

first insemination suggested that embryonic mortality occurred just before 42 days of fertilization of the ova. The major reason for discrepancies in diagnosis of pregnancy with milk progesterone appeared to be due to normal variation of the estrous cycle (short or long) and embryonic mortality (Ax, 1980 and Gowan *et al.,* 1981).

Table (4): Individual changes in milk progesterone (ng/m) over 42 days after the first fertile service (FFS) in Egyptian buffaloes (Group 4).

Days after FFS	Buffalo Number						Aver. ± SE
	1	2	3	4	5	6	
0	0.00	0.00	0.00	0.96	0.00	0.00	0.16[f]
2	0.01	0.00	0.31	1.69	0.01	2.09	0.68 ± 0.39 [f]
6	1.12	2.05	5.99	2.14	1.77	3.39	2.74 ± 0.38[ef]
10	2.04	6.97	3.56	0.00	5.03	4.61	3.70 ± 0.72[def]
14	25.18	14.21	15.07	17.17	10.11	10.49	15.37± 1.00[a]
18	17.92	10.34	10.84	18.03	3.96	5.46	11.09± 2.25[abc]
20	12.50	23.34	12.68	12.75	4.17	6.67	12.02±2.70[ab]
22	10.59	7.91	7.11	6.63	2.14	3.96	6.39 ± 1.17[cde]
25	10.44	20.81	5.15	7.09	7.73	3.20	9.07 ± 2.55[bcd]
42	13.99	10.18	6.39	7.29	3.39	4.88	7.69 ± 1.57[bcde]
Aver. ±SE	9.38 ±2.70	9.58 ±2.25	6.71 + 1.57	7.37 ±2.09	3.83 ±1.01	4.47 ±0.89	6.90 ±0.81

Means followed by different letters are significantly differed (P <0.01)

Jain (1989) reported that the plasma progesterone concentration did not exceed 0.4 ng/ml at day of estrus, then it reached a peak of 4.0 ng/ml at day 11 after insemination and maintained a plateau up to day 15. Batra *et al.* (1979) showed that the MP concentration in pregnant Murrah buffaloes was 3.5, 17.3, 21.2 and21,9 ng/ml on days 3, 15, 18 and 21 after the fertile service, which are comparatively greater than that obtained in the present study (Tables 3 and 4). This may be due to breed differences and buffalo milk composition as well as procedure of sampling. Schiavo *et al*. (1975) found that the first milk contained 3.69 ng/ml progesterone on days 10 and 13 of the cycle; meanwhile, the last milk of the same animal was 7.99 ng/ml. Similarly, progesterone in the first and last milk was 5.08 and 9.57 ng/ml at days 13-16 of the cycle, respectively.

The obtained data (Tables 3 and 4) clearly indicated that the MP on days 20, 21, 22, or 25 after service could be a useful tool for pregnancy diagnosis. The results could be more beneficial, if the assay was conducted or double samples with 2-3 days apart between the periods from day 20 to 25 after service. This finding agrees with that obtained by Arora *et al*. (1979) in buffaloes and Ginther *et al*. (1974) in cattle. These investigators reported that MP at day 20 to 24 post breeding could be used as a tool for early estimate of pregnancy in buffaloes and cattle, respectively.

Pennington *et al.* (1985) stated that total accuracy of cows diagnosed as being pregnant or non – pregnant by MP on day 24 post breeding was greater than diagnosis on day 21 post breeding, when cows in estrus by day 24 were deleted from the data, similarly, Cox *et al.* (1978), AX (1980) and Gowan *et al*. (1981) reported that days 20 to 24 post breeding appear best for single sample for pregnancy diagnosis using MP . In a previous study, Pennington *et al*. (1976) found that accuracy of pregnancy diagnosis with milk progesterone tented to be greater on day 21 than on day 24.

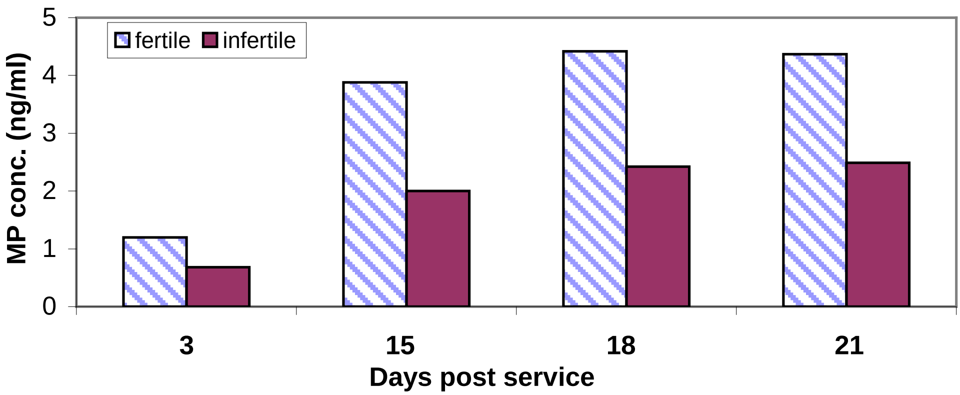

Fig. (1): Milk progesterone concentration (MP) on days 3, 15, 18 and 21 post fertile and infertile services in buffaloes.

As shown in Tables (2, 3 and 4) and Fig. (1), the fertile inseminations were accompanied with higher MP concentration on days 3, 15, 18 and 21 post service than that were accompanied with the infertile inseminations. Rosenberg *et al*. (1977) reported that in summer infertile inseminations were preceded by a cycle in which the mean of progesterone concentration were significantly lower. Recently, El-Sobayil *et al*. (2007) reported that overall concentration of milk fat progesterone throughout days 6 and 3 before estrous and days 1, 3 and 6 after estrous was significantly ($p<0.05$) higher in conceived than non-conceived buffaloes (82.2 vs. 27.1 ng/ml). Also, El-Moghazy *et al*. (2006) stated that concentration of milk progesterone throught

postpartum first ovulation interval in conceived buffaloes was significantly higher (0.57 ng/ml) than that in non-conceived ones (0.34 ng/ml).

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إستخدام هرمون البروجسترون اللبن كدليل لمعرفة الحالة التناسلية فى الجاموس المصرى

عبد الله فتحى نيبر[1] ، حلمى عبد الرحمن عبد الهادى[1] ،
السعيد النعيمى حبيبب[2] ، وريم صابر مراد[1]
١- قسم الإنتاج الحيوانى – كلية الزراعة – جامعة المنوفية.
٢- قسم التطبيقات البيولوجية– هيئة الطاقه الذرية– إنشاص.

الملخص العربى

تم جمع عدد ١٧٩ عينة لبن من ٢٧ جاموسة تم تقسيمها إلى أربع مجموعات حسب حالتها التناسلية لدراسة إمكانية مدى إستخدام هرمون بروجسترون اللبن للتعرف على الحالة التناسلية فى الجاموس المصرى. إشتملت المجموعة الأولى على عدد ٧ جاموسة ممثلة لمرحلة إسترداد الرحم لحجمه الطبيعى عقب الولادة وفترة ا للاشياع ، وتم أخذ عينات اللبن من اليوم ١٩ إلى اليوم ٤٧ بعد الولادة. واشتملت المجموعة الثانية على عدد ٧ جاموسة تم تلقيحها عند ظهور أول شياع بعد الولادة ، و بعد التلقيح لم تظهر على هذه الحيوانات أى علامات للشياع وتم إجراء الجس المستقيمى بعد ٦٠ يوم من التلقيح واتضح أن جميعها فارغة مع وجود بعض التراكيب المبيضية الشاذة ، وتم جمع عينات اللبن من هذه المجموعة فى اليوم ٣ ، ١٥ ، ١٨، ٢١ عقب التلقيح. اشتملت المجموعة الثالثة على عدد ٧ جاموسة لتقدير التغيرات فى تركيز هرمون البروجسترون باللبن عقب التلقيح المخصب وتم أخذ عينات اللبن فى اليوم ٣ ، ١٥ ، ١٨ ، ٢١ بعد التلقيح. كما اشتملت المجموعة الرابعة على عدد ٦ جاموسة أخرى لتقدير التغيرات فى تركيز هرمون بروجسترون اللبن ولكن فى الأيام صفر، ٢ ، ٦ ، ١٠ ، ١٤ ، ١٨ ، ٢٠ ، ٢٢ ، ٢٥ ، ٤٢ عقب التلقيح المخصب.

وقد أظهرت النتائج ان متوسط تركيز هرمون بروجسترون اللبن فى فترة إستعادة الرحم لحجمه الطبيعى كان ٠.٩١ ± ٠.١٨ نانوجرام / مل تتراوح بين صفر – ٨.٢٥ نانوجرام / مل. كما أوضحت النتائج أن مستوى هرمون البروجسترؤن فى عينات اللبن التى تم جمعها فى الأيام ٣

، ١٨.١٥ ، ٢١ بعد التلقيح المخصب لحيوانات المجموعة الثالثة أعلى منها فى عينات اللبن لحيوانات المجموعة الثانية ذات التلقيح الغير مخصب (١.٢ ، ٣.٨٨ ، ٤.٤٢ ، ٤.٣٧ مقابل ٠.٦٨ ، ٢.٠ ، ٢.٤٢ ، ٢.٤٩ نانو جرام/مل ، على التوالى). وأتضح من الفحص التناسلى لحيوانات المجموعة الثانية وجود بعض التراكيب المبيضية الشاذة (٤٢.٩% أظهرت إستدامة الجسم الأصفر ، ٢٨.٦% ذات مبايض ملساء ، ١٤.٣% بها مبيض متحوصل، ١٤.٣% ذات مبيض عادى التركيب). وقد اشار إرتفاع مستوى هرمون بروجسترون اللبن فى اليوم ٢١ لحيوانات المجموعة الثانية إلى تشخيص الحمل بصورة غير صحيحة لحوالى ٥٧.١% من حيوانات هذه المجموعة. إرتفع مستوى هرمون بروجسترون اللبن بعد التلقيح المخصب فى حيوانات المجموعة الرابعة بصورة معنوية (على مستوى ١%) من ٠.١٦ نانو جرام/مل فى يوم التلقيح حتى وصل أقصاه (١٥.٣٧ نانو جرام /مل) فى اليوم ١٤ بعد التلقيح ، وظل بعد ذلك فى مستوى أعلى من ٦.٠ نانوجرام/مل حتى اليوم ٤٢ بعد التلقيح. كما أوضحت نتائج هذه الدراسة أنة يمكن استخدام تركيز هرمون البروجسترون فى اللبن لتشخيص الحمل فى الجاموس بدرجة دقة ٨٦% . وبالتالى يستخلص أنة يمكن استخدام تركيز هرمون البروجسترون فى اللبن كدليل لمعرفة الحالة التناسلية فى الجاموس كتشخيص الحمل (بدرجة دقة ٨٦%) أو لمعرفة النشاط المبيضى. ومن الأفضل من الناحية الاقتصادية إجراء تقدير هرمون البروجسترون فى اللبن فى اليوم ٢٤ بعد التلقيح .