

PERIPARTUM CONCENTRATIONS OF ESTRADIOL AND PROGESTERONE IN BLOOD OF PRIMIPAROUS BUFFALOES AS INDICATOR OF PROSPECTIVE POTENCY OF MAMMARY GLAND

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SUMMARY

Levels of estradiol (E2) and progesterone (P4) in the peripheral blood of nine Egyptian primiparous buffaloes were assessed in peripartum short period. The animals were divided into three equal groups according to their lactation period length (LP) being, G1 (≥ 9 months), G2 (≤ 7 months) and G3 (non-lactating group). The results of G1, showed increased concentration of E2 continued till it reached its zenith 2 days prior to calving. The rate of increasing E2 level in that time was greater by 110.3% in G1 than that in G2. After calving, E2 concentration decreased till the 5th day then increased at the 7th day in G2, while, in G1 there was an increase at the days 2 and 7 with a drop on the 5th day. Concentration of E2 was considerably lower (21.95 ± 15.01 pg/ml) in G3 two days before calving in comparison with that of G1 (200.39 ± 15.01 pg/ml) and G2 (95.30 ± 15.01 pg/ml). NO detectable differences were observed in concentration of P4 among the studied groups, particularly during the precalving period. Lactation period was longer in G1 than that in G2 (285 vs. 156 days), however, the daily milk yield showed the opposite trend. It was slightly greater in G2 than that in G1 (6.4 vs. 6.0 kg).

It is concluded that, peripartum pattern of change in the concentration of E2 may have a good indication for the prospective potency of lactation performance of primiparous buffaloes. The depression in the E2 concentration in the precalving period seems to have a negative effect on postpartum mammary gland activity.

Keywords: *Estradiol, progesterone, primiparous buffaloes, milk production*

INTRODUCTION

The endocrine system, perhaps rather than any other physiological system plays a central role in all aspects of mammary gland development (Akers, 2006). The level of reproductive hormones, including: estrogen (E2), progesterone (P4), placental lactogen (PL), prolactin (PRL) and oxytocin (change with the reproductive state) all have a direct action on mammary gland development (Neville *et al.*, 2002). Estrogen alone stimulates mammary ducts proliferation, while normal lobule-alveolar

development requires synergistic effects of both E2 and P4 (Neville *et al.*, 2001 & 2002 and Divakar *et al.*, 2008). During the following gestation time, concentration of E2 either remains high or continues to increase and PRL is markedly elevated in blood, while blood flow rate of mammary gland increases. Prepartum extent of transfer of hormones to accumulating secretions in cows is unknown. Moreover, their presence in alveolar-lumina during precalving and lactation periods may have important effects on onset of lactation (Keller *et al.*, 1977).

Recently, more attention has been focused on dairy buffalo because there are many obstacles, severely, affecting its reproductive and lactational performance. Abruptly cessation of milking after calving represents one of those obstacles that affect economic importance of buffalo raising. Ilieva and Peeva (2007) found that the highest percentage of culled buffalo cows was recorded after their first lactation. Meanwhile, Afzal *et al.* (2007) reported that in order to develop productivity of dairy buffalo it is necessary to develop understanding the factors affecting its milk production. Omar *et al.* (1994) stated that in Egyptian dairy buffalo herds, some buffaloes calved and failed to lactate or stopped milking after a very short time. Also, it was found according to the farmal records that about 44.4% of primiparous buffaloes in EL-Nattaf farm were non-lactating after calving.

This study aimed to assess the levels of estradiol and progesterone hormones during precalving and postcalving short periods, in relation to prospective lactation potency of Egyptian primiparous buffaloes.

MATERIALS AND METHODS

This work was carried out at El-Nattaf El-Kadeem farm (Kafr El-Sheik governorate which located in the northern part of the Nile Delta. This farm belonging to Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Dokki, Giza, Egypt. This study was lasted for 20 months from the beginning of September, 2006 till the end of April, 2008.

Experimental animals:

Nine primiparous buffaloes were assigned at their late pregnancy (2 months before calving) and were closely monitored specially at the days near to the expected day of calving. The animals calved during the period from November, 2006 till July, 2007 after gestation period (lasted 307.8 days on average). The average of age at first calving (AFC) for all heifers was 33.88 months and average of body weight (BW) at calving was 359.17 kg for the lactating buffalo (LB) groups (G1 and G2), but the non lactating buffalo (NLB) group (G3) was the lowest and ranged between 300 to 312 with an average of 307.33 kg. In addition, the average birth weight of born calves from G1 and G2 groups was 30.5 kg, while that in G3 was 16.67 kg and all calves in G3 were died after calving by 3 days. These buffalo heifers were housed in shaded open yards. The experimental animals were fed according to the recommendations of APRI. The concentrate ration included; 9.65% moisture, 7.12 % ash, 16.15% protein, 2.28% fat and 11.17% fiber. Rice straw was available to each buffalo *ad libitum*. The nutrient requirements were adjusted according to BW, pregnancy status, and subsequent milk production of each buffalo. After calving, these buffaloes were hand milked for one week and then machine milked twice daily at 5:00 h and 17:00 h.

Experimental design:

The experiment lasted till the buffaloes were dried and stopped in milking, then buffaloes were divided into three equal groups according to their lactation period length into G1 (that lactate for ≥ 9 months), G2 (that lactate for ≤ 7 months) and G3 (that failed to lactate after calving). Averages of total milk yield (TMY) and length of lactation period (LP) were determined and recorded. Whereas, the average of daily milk yield (DMY) was calculated. The time of recording milk yield preceded the time of blood sampling on the days of blood collection.

Blood samples (10 ml) were obtained from each animal at 7, 5, and, 2 days precalving, on day of calving (0 day) and at 2, 5, and 7 days postcalving. Blood samples were collected from the Jugular vein in heparinized tubes at 9.0 am before feeding and watering. Samples were centrifuged at 3000 r.p.m for 20 minutes to separate clear blood plasma, then stored at (-20°C) until the hormonal assay using the radioimmunoassay (RIA) technique was executed. The assays were executed at the hormonal analysis unit, Faculty of Agriculture Research Park, Cairo University, Egypt.

Estradiol determination:

A single antibody RIA technique was used to quantify plasma estradiol (E2) using Coat-A-Count kit supplied by Siemens® Medical Solutions Diagnostics, Los Angeles, CA 90045-6900, USA. The used antiserum is highly specific for estradiol with 5.34 intra-assay precision and 6.4 inter-assay precision. The analytical sensitivity is 8 pg/ml.

Progesterone determination:

A single antibody RIA technique was used to assay plasma progesterone (P4) using Immunotech kit, supplied by A Beckman Coulter Company, Mareseille, France. The used antibody has extremely low cross reaction against other naturally occurring steroids. The intra-assay is $\geq 5.8\%$ and the inter-assay is $\geq 9.0\%$. The analytical sensitivity is 0.05 ng/ml.

Statistical analysis:

Data were analyzed for blood plasma hormones using Repeated Model procedure (SAS, 1999). Significance among means was tested according to Duncan (1995). The model was as follow:

$$Y_{ijk} = \mu + TRT_i + T_j + TRT_i * T_j + e_{ijk}$$

Where:

μ = Overall mean

TRT_i = Effect of groups, $i = 1, 2, 3$;

T_j = The effect of pericalving period, $j = -7, -5, -2, 0, +2, +5, +7$;

$TRT_i * T_j$ = Interaction between pericalving (T) and the groups.

e_{ijk} = The effect of random error.

RESULTS AND DISCUSSION**Estradiol:**

A successful lactation in the dairy cow requires the mammary gland to produce a large number of potential milk secreting cells during pregnancy. These cells subsequently must differentiate during the periparturient period such that the mammary epithelium develops the biochemical and cytological machinery to synthesize and secrete milk. Both mammary growth and initiation of milk synthesis

are intimately dependent upon complex interactions among hypophyseal, adrenal, ovarian, and placental hormones (Tucker, 1979; Cowie *et al.*, 1980; Delouis *et al.*, 1980; Elias, 1980.; Topper and Freeman, 1980.; and Tucker, 1981).

In the present study, Table (1) and Fig. (1) revealed that the concentrations of estradiol (E2) in G1 and G2 were increased from 7 days precalving and continued to increase till it reached its maximum at 2 days precalving. These maximum concentrations were 200 and 95 pg/ml in G1 and G2, respectively. The concentrations declined precipitously on the day of calving, in the two groups, and continued decreasing, on average, to reach the minimum value the fifth day postcalving followed by a considerable increase on the 7th day postcalving. This enormous rise in the concentration of E2 before calving in G1 enhances lactation performance to longer duration and greater total milk yield as recorded in Table (4). Smith *et al.* (1973) found that the first major change in serum steroids hormones was a 10-fold increase in estrogens during the month before parturition, to a peak at about 2 days before parturition. In this respect, previous studies (Convey, 1974 and Tucker, 1981) also confirmed that the E2 concentration continued increasing within 2 weeks prior calving and reached its zenith 2 days before calving. Erb (1976) clarified the role of E2 in milk secretion and stated that E2 continues to increase until day of calving and may stimulate pituitary release of PRL and enhance rate of milk secretion or perhaps synergize mechanisms required for onset of normal lactation, such as functions of corticoids, growth hormone and other factors in blood plasma. Smith *et al.* (1973) found that glucocorticoids peak at parturition and return to basal values within 12 hrs. and remained near that basal levels for 9 days postpartum. The present results are slightly different from those reported by Henrichs *et al.* (1972), Chew *et al.* (1977), Erb *et al.* (1981) and Kaker *et al.* (1984) who stated that the peak of E2 level in cattle breeds is on the day of calving not before calving by 2 days, however they stated that the values decline thereafter. It is clear in Table (1) and Fig. (1) that G3 during precalving period had low levels of E2 concentration, especially at 2 days precalving. According to the previous studies (Turner and Bagnara, 1971; Convey, 1974; Tucker, 1981 and Tucker, 2000), it could be deduced that the lower levels of E2 during the precalving period may make these heifers loss the role of E2 in; increasing the enzymatic activity in the secretory cells to be ready to the onset of lactation. E2 acted in at least two ways to initiate lactation: 1) in several species including cattle, it caused release of PRL (which is critically important for initiation of lactation in the periparturient period) from the anterior pituitary gland into blood, 2) increasing the number of PRL receptors in mammary cells, which is another lactogenic event (Tucker, 2000). Figure (2) shows that the cocentration of E2 in LB (G1 and G3) was higher than that in NLB (G3) during the different pericalving periods, particularly during the different precalving periods by about 91.6%.

Progesterone:

Progesterone (P4) concentrations during pericalving stages are shown in Table (2) and Figure (3). At 7 days precalving, the P4 level (ng/ml) in G1 was markedly lower (1.84) than that in G2 (3.14) and that in G3 (3.07). This lowest level in G1 may help in preparation of the mammary gland for lactation. It is clear that G2 showed the highest values of P4 at 7 and 5 days prior calving compared with those in G3 and particularly in G1. From 7 to 2 days before calving, P4 level decreased progressively in G2 whereas it was dropped dramatically in G3. In contrast, these changes in G1

were slight to fulfill maintenance of lactation potency (Figure 3). Therefore, it could be suggested that the sharp changes in pre-calving P4 level in both G2 and G3, particularly in G3, may be responsible for the low lactation potency in these groups. From 5 to 2 days before parturition, P4 level slightly increased (9%) in G1. On the other hand, it was greatly decreased in both G2 (43%) and G3 (64%). During the last 2 days of pregnancy, just prior calving, all heifers decreased their P4 level at calving time. This reduction coincides with the last phase of lactogenesis.

Table 1. Estradiol concentrations (pg/ml) in nine Egyptian primiparous buffaloes from 7 days before to 7 days after calving (LSM ± S.E.)

Group	Precalving		Calving		Postcalving		Average	
	-7	-5	-2	0	+2	+5		+7
G1	95.0 ±15.0	134.23 ±15.01	200.39 ±15.01	31.80 ±15.01	37.55 ±15.01	17.40 ±22.93	35.36 ±15.01	81.90 ^a
G2	85.7 ±31.0	82.32 ±15.01	95.30 ±15.01	39.43 ±30.95	19.04 ±15.01	19.04 ±15.01	32.65 ±15.01	53.96 ^b
Average	90.4	108.27	147.84	35.61	28.29	18.22	34.01	66.08
G3	110.1 ±21.2	48.82 ±15.01	21.95 ±15.01	23.05 ±21.23	14.15 ±21.23	27.35 ±21.23	19.95 ±21.23	39.509 ^b
Overall mean	97.9 ^a	88.46 ^a	105.89 ^a	30.35 ^b	29.82 ^b	21.48 ^b	31.75 ^b	57.94

G1= Group that milking for more than 9 months, G2 = Group that milking less than 7 months and G3 = Group with no lactation. Means with different letters in the same column and row are significantly different at 0.05 level of probability.

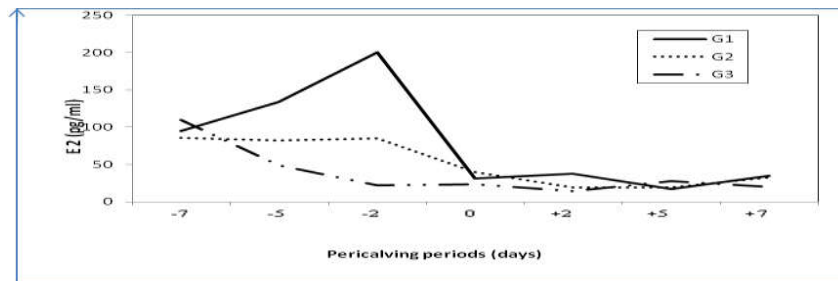


Fig. 1. Average estradiol (E2) concentrations from 7 days before to 7 days after calving in primiparous buffaloes that lactating for more than 9 months (G1), that lactating less than 7 months (G2) and those with no lactation (G3)

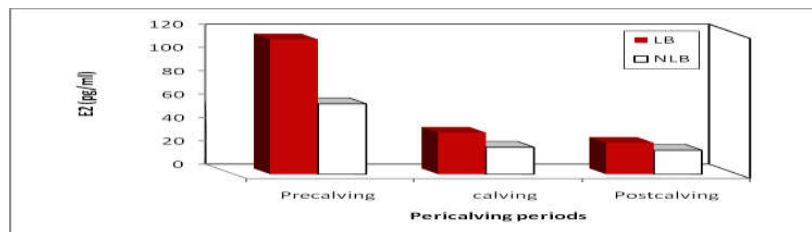


Fig. 2. Average estradiol (E2) concentration during one week before to one week postcalving in lactating (LB) and non- lactating (NLB) primiparous buffaloes

At calving, G1 and G3 showed the lowest level of P4 which was one-third of that in G2. At that time, level of P4 dropped in G1 to one-fifth of that at 2 days prior calving. However, it was decreased slightly in G2 and almost unchanged in G3.

During the post-calving period, the changes in p4 level were less pronounced than those during pre-calving period. It was slightly fluctuated in G1. Whereas, it was decreased progressively in G2 and increased successively in G3. Therefore, it could be noticed that P4 level at 7 days post calving in G1 and G2 (LB) showed opposite trend to that in G3 (NLB). It was decreased in G1 and G2 and increased in G3.

It is obvious that during the periparturient period, the changes in P4 level agree well with the inhibitory effects of this hormone on lactogenesis as observed in G3. The present results are in agreement with those of Short (1958); Stabenfeldt *et al.* (1970); Smith *et al.* (1973); and Tucker (1981) who stated that there was a decrease in P4 secretion 1 to 2 days before calving in preparation of the onset of lactation. This activity is inhibited by high concentration of P4, the primary inhibitor of lactation (Erb, 1976 and Akers, 1985). In addition, P4 competes with glucocorticoids receptors and prevents them from initiating lactation. The parturient rise in glucocorticoids occurred shortly after the fall in P4 (Smith *et al.*, 1973). Furthermore, P4 blocks the ability of PRL to induce synthesis of PRL receptors (Tucker, 1981). As shown in Fig. (4), P4 concentration was higher by 35.8% in LB (G1 and G2) than that in NLB (G3) during precalving period.

Table 2. Progesterone concentrations (ng/ml) in nine Egyptian primiparous buffaloes from 7 days before to 7 days after calving (LSM \pm S.E.)

Groups	Precalving			Calving		Postcalving		Average
	-7	-5	-2	0	+2	+5	+7	
G1	1.84 ± 0.43	1.43 ± 0.43	1.56 ± 0.43	0.34 ± 0.43	0.38 ± 0.43	0.69 ± 0.66	0.45 ± 0.43	1.52 ^a
G2	3.14 ± 0.89	2.74 ± 0.43	1.56 ± 0.43	1.13 ± 0.43	1.04 ± 0.89	0.75 ± 1.09	0.61 ± 0.43	1.52 ^{ab}
Average	2.49	2.08	1.56	0.73	0.71	0.72	0.53	1.20
G3	3.07 ± 0.61	1.07 ± 0.43	0.39 ± 0.43	0.35 ± 0.43	0.41 ± 0.43	0.47 ± 0.66	0.55 ± 0.87	0.78 ^b
Overall mean	2.09 ^a	1.74 ^a	1.20 ^{ab}	0.44 ^b	0.49 ^b	0.63 ^b	0.52 ^b	1.01

G1= Group that milking for more than 9 months, G2 = Group that milking less than 7 months and G3 = Group with no lactation.

Means with different letters in the same column and row are significantly different at 0.05 level of probability

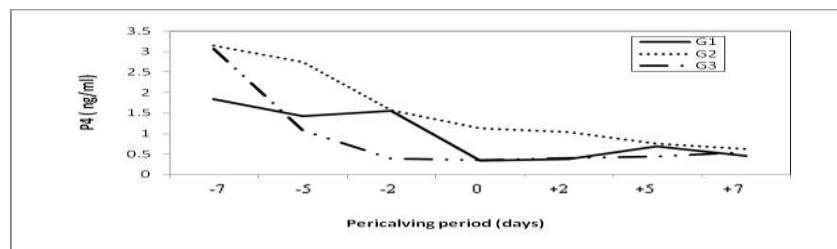


Fig. 3. Average progesterone (P4) concentrations from 7 days before to 7 days after calving in primiparous buffaloes that lactating for more than 9 months (G1), that lactating less than 7 months (G2) and those with no lactation (G3)

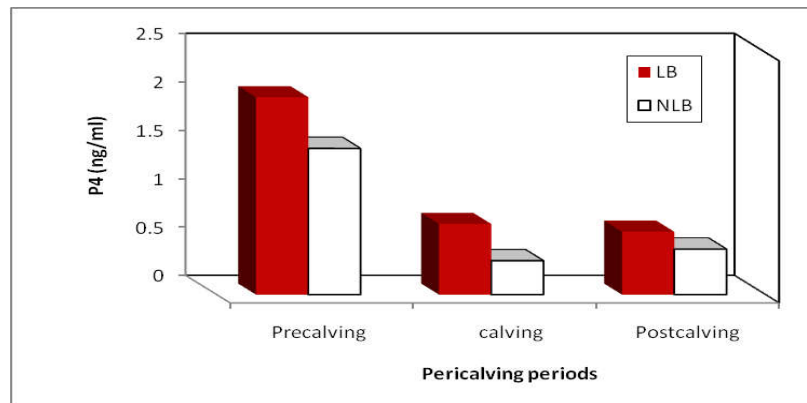


Fig. 4. Average progesterone (P4) concentration during one week before to one week postcalving in lactating (LB) and non-lactating (NLB) primiparous buffaloes

Progesterone-estradiol ratio:

Comparing P4 and E2 levels in non-lactating (NLB, G3) group with that in the lactating groups (LB, G1 and G2), it was observed that both had higher values in LB than that in NLB during pre-and-post calving periods (Figurs, 2 and 4). The lower levels of P4 and E2 in G3 may indicate hormonal imbalance leading to improper physiological functions related to onset of normal delivery and initiation of milk secretion as well. The rate of decline in P4 concentration from pre-to-post calving period was 68.3% in LB vs. 68.2% in NLB. While, that of decline in E2 was 76.8% in LB vs. 66.0% in NLB. In lactating groups, P4/E2 ratios were 17.75 and 24.22 in pre and post calving, respectively, while, those in NLB were 25.05 and 23.44%, respectively (Table 3). This finding indicates that prepartum level of P4 was abnormally greater or that of E2 was abnormally lower in NLB so that greater P4/E2 ratio (25.05%) attained which is not in favor of milk secretory mechanism.

Table 3. Average of estradiol (E2), progesterone (P4) and ratio of P4/E2 in lactating groups (G1 and G2) and non-lactating group (G3) during peripartum period

Hormone	Lactating (LB)		Non-lactating (NLB)		Rate of decline	
	Prepartum	Postpartum	Prepartum	Postpartum	LB	NLB
E2	115.49	26.84	60.29	20.48	88.65	39.81
P4	2.04	0.65	1.51	0.47	1.39	1.04
P4/E2	17.66	24.22	25.05	22.95	-6.55	2.1

Generally, the three experimental groups exhibited a downward trend of P4 concentration during prepartum period until day of calving. However, concentrations of P4 were relatively higher in G2 than that in G1 and G3 during prepartum period and day of calving. The overall mean of P4 concentration during postpartum period was significantly less than that during the prepartum period for all studied groups and it was relatively greater in G2 than other groups.

Lactation potency:

Milk production is largely dependent on the shape of lactation curve. The key element that describes the pattern of milk is peak yield, which represent the maximum output during the lactation and its persistency. Bussi and Sala (1993) stated that lactation period in buffalo is less than cattle (250 vs. 300 days) and the total amount of milk production in buffalo is less than cattle (2000 kg vs. 7.200 kg) and as mentioned by Meikle *et al.* (2004), the primiparous cattle are producing milk less than multiparous cattle and they attributed that to mammary gland which is more metabolically active in multiparous than primiparous cow and the DNA content of mammary secretory cells is lower in primiparous than multiparous. In the present study, as shown in Table (4) the average of lactation period length in G1 (285 days) was longer than that in G2 (156 days) by 82.7%. The average of the total milk yield in the two groups (G1 and G2) is lower than that recorded by Aziz and Badran (1993) who found that the amount of total milk yield in Egyptian buffaloes is 1669 kg. In addition, the average of daily milk yield (DMY) of lactating primiparous buffalo in the present study was higher than that recorded by Barbosa *et al.* (2007) and Abou-Ziena *et al.* (2009) who recorded that the average DMY in buffaloes was 3.51 and 3.97 kg, respectively. The lactation performance of these primiparous buffaloes as presented in Table (4) could be attributed to the levels of E2 especially before calving by 2 days as discussed previously which was higher in G1 than G2 by 110.3% than that in G3, this may help the mammary gland of these buffalo heifers to develop more during lactation through the effect of E2 on releasing prolactin. In addition, the heifers of G2 were slightly hard in milking compared with the heifers in G1. This may make this group losing the role of milking in stimulating prolactin release from pituitary gland, as reported by Akers (1985) who stated that milking or even teat stimulation causes release of prolactin.

Table 4. Lactation period (LP), total milk yield (TMY) and daily milk yield (DMY) in primiparous buffaloes that milking more than 9 months (G1) and those milking less than 7 months (G2)

Group	Buffalo	LP	TMY	DMY	
		(day)	(kg)	Mean±SE	Range
G1	1	286	1866	7.0±0.6	2 - 12
	2	290	1905	6.2±0.5	2- 10
	3	279	959	4.8±0.4	1- 8
	Average	285	1576	6.01	1.6 – 10
G2	4	84	782	8.3±0.7	6 - 11
	5	199	1541	7.3±0.7	2 – 10
	6	185	654	3.7±0.3	2 - 7
	Average	156	992	6.44	3.3 – 9.3

CONCLUSION

Apart from the involvement in reproductive functions, changes in each of E2 and P4 concentrations in this study are synchronized for mammary gland development and prospective potency of lactation. It is well known that E2 alone stimulates duct proliferation but normal lobule-alveolar development requires synergistic effects of E2 and P4. This action is secured by drop of P4 level concomitant with high level of

E2 as found in G1 alongside in comparison with G2. It is clear that G3 which had a very low concentration of E2 and a considerable high concentration of P4 failed to produce milk. This synergistic action is fulfilled by: a) increased permeability of cellular membrane, b) accumulation of fluid in extracellular spaces, increased vascularity and, presumably, increased blood flow rate and c) stimulation of RNA-directed synthesis of cellular proteins in the secretory cells. The present results revealed that additional researches will be necessary to clarify the interaction of estradiol and progesterone prior to parturition. Also, monitoring of the hypophyseal, adrenal, ovarian and placental hormones simultaneously will be required to complete understanding of parturition and lactogenesis in primiparous buffalo under good managerial conditions and on large numbers.

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تركيز هرموني الإستراديول والبروجستيرون حول الولادة في دم العجلات الجاموسى كمؤشر على القدرة المتوقعة لنشاط الغدة اللبنية

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تم تقدير مستويات هرموني الإستراديول والبروجستيرون في دم العجلات الجاموسى خلال فترة قصيرة حول الولادة. حيث تم تقسيم الحيوانات إلى ثلاث مجموعات متساوية تبعاً لطول فترة الحليب. فكانت هذه المجموعات كالتالى: المجموعة الأولى (أكثر من ٩ شهور)، المجموعة الثانية (أقل من ٧ شهور) أما المجموعة الثالثة (الغير حلابية).

وأظهرت نتائج المجموعة الأولى زيادة مستمرة في تركيز هرمون الإستراديول حتى وصل إلى أقصى تركيز له قبل الولادة. وكان معدل الزيادة في تركيز هرمون الإستراديول عند ذلك الوقت أعلى بحوالي ١١٠.٣% في المجموعة الأولى عن الثانية. أما بعد الولادة إنخفض تركيز هرمون الإستراديول حتى اليوم الخامس ثم ارتفع مستواه عند اليوم السابع في المجموعة الثانية. أما المجموعة الأولى أظهرت زيادة في مستواه عند اليوم الثاني والسابع بينما كان هناك إنخفاض في اليوم الخامس. عند اليوم الثاني قبل الولادة كان هناك إنخفاض ملحوظ في تركيز هرمون الإستراديول (٢١.٩٥ بيكوغرام/مل) في المجموعة الثالثة مقارنة بتركيزه في كل من المجموعة الأولى (٢٠٠.٣٩ بيكوغرام/مل) والمجموعة الثانية (٩٥.٣٥ بيكوغرام/مل). على الجانب الأخر لم توجد أي إختلافات واضحة في تركيز هرمون البروجستيرون بين حيوانات المجموعات الثلاثة خاصة أثناء الفترة قبل الولادة. وكانت فترة الحليب أطول في المجموعة الأولى عن المجموعة الثانية (٢٨٥ مقابل ١٥٦ يوم) بالرغم من أن إنتاج اللبن اليومي أظهر عكس ذلك حيث كان أعلى في المجموعة الثانية عن المجموعة الأولى (٦.٤ مقابل ٦.٠ كجم).

ويمكن أن نستنتج من هذه الدراسة أن التغير في تركيزات هرمون الإستراديول حول الولادة يعتبر مؤشر جيد على القدرة المتوقعة للغدة اللبنية على كفاءة إنتاج اللبن للعجلات الجاموسى. ويبدو أن الإنخفاض في مستوى هرمون الإستراديول أثناء الفترة قبل الولادة يؤثر تأثيراً سلبياً على نشاط الغدة اللبنية بعد الولادة.