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Ovarian activity and hormonal relationship in pregnant buffaloes

Waheed M. M.

Department of Clinical Studies, Faculty of Veterinary Medicine and Animal Resources, King Faisal University, Kingdom of Saudi Arabia

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SUMMARY

The morphological characters of ovaries were studied in fifty one slaughtered Egyptian pregnant buffaloes including the weight and diameter of corpora lutea, and the number and size of follicles. Progesterone and estradiol-17ß concentrations were estimated in both peripheral plasma and feces using Enzymimmunoassay. The results revealed a decrease in number of all sized follicles as pregnancy advanced. Plasma and fecal progesterone concentrations increased and plasma estradiol-17ß levels decreased with the progress in pregnancy. Positive Correlation Coefficients existed between the ovarian weight and weight of corpora lutea in one side, and between the number of follicles and estradiol-17ß levels on the other side. The Correlation Coefficients between the weight of corpora lutea and estradiol-17ß levels were negative. Seasonal differences in ovarian activity occurred in pregnant buffaloes coincided with the profound effect of season on the progesterone and estradiol-17ß concentrations in the peripheral plasma.

Keywords: Buffalo, Pregnancy, Ovary, Progesterone, Estradiol-17ß

INTRODUCTION

Despite buffalo importance in animal production industry, there are a relative few number of studies regarding its physiology (Martin *et al.*, 2008). Relatively little references exist about the morphological and physiological aspects of buffalo ovary during pregnancy. In pregnant cows, corpora lutea on an ovary may modify the pattern of growth and atresia of follicles on the same ovary (Rexroad and Casida, 1975). Cows during pregnancy continue to manifest follicular waves at intervals of 8 to 10 days (Rexroad and Casida, 1975; Pierson and Ginther, 1987; Ginther *et al.*, 1989; Taylor and Rajamahendran, 1991). The follicular development is characterized by waves of different patterns in pregnant buffaloes and

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new studies with hormone assays could be more elucidative (Martin *et al.*, 2008).

The objective of the present study was to measure the ovarian activity of pregnant buffaloes in form of morphological evaluation of corpora lutea and follicles as well as determination of progesterone and estradiol-17ß concentrations in both peripheral plasma and feces of these buffaloes.

MATERIALS AND METHODS

Animals:

A total of fifty one pregnant, healthy Egyptian buffalo-cows (Bubalus bubalis) aged from 5 to 10 years old were used. These buffaloes were slaughtered at El-Warak slaughter-house, Giza, Egypt. Pregnancy was diagnosed per rectum before slaughter. Immediately after slaughter, the genital organs were removed, the ovaries were maintained intact and transported to the laboratory. In the laboratory, the reproductive organs were dissected and stages of pregnancy were determined (Abdel Raouf and El Naggar, 1968; Luktuke, 1983).

Ovaries were weighed to the nearest mg. All the follicles on the surface of the ovaries were counted and their diameter was measured using a pair of vernier calipers. Follicular diameter was partitioned into three discrete size groups: [a] small (< 6mm), [b] medium (6-10mm) and [c] large (>10mm; Schmidt *et al.*, 1963; El-Wishy, 1965; Brantmeier *et al.*, 1987). The corpora lutea were dissected free of extraneous tissue and their diameter and weight were recorded.

Blood and fecal samples:

At the time of rectal palpation, fecal samples were collected, transported to the laboratory and stored at -20°C pending analysis. Blood samples (10 ml) were collected from the jugular vein at slaughter in labeled heparinized tubes (5 IU heparin/tube). In the laboratory, the blood samples were centrifuged at 14,000 g for 10 minutes at 4°C. Blood plasma was stored at -20°C until analysis.

Progesterone and estradiol – 17 ß Enzymimmunoassay (EIA):

Progesterone and estradiol-17ß were determined in the peripheral blood plasma and feces of pregnant buffaloes by Enzymimmunoassay (ELISA). In brief, progesterone and estradiol-17ß were extracted from feces by adding 0.5 ml distilled water and 4.0 ml of absolute methanol to 0.5g of feces. The mixture was shaken for 30 minutes to remove lipids then 3ml of petroleum ether was added. After thorough mixing in a vortex for 10 seconds, the mixture was centrifuged (1500g) for 10 minutes. The methanol and ether layers were separated by cooling at 20°C for one hour. The methanol extract was drown and stored at -20°C pending analysis (Panchal et al., 1992; Palme and Mostle, 1993; Palme *et al.*, 1993). Blood plasma and fecal concentrations of progesterone and

estradiol were determined by ELISA (Absorbance Microplate Reader ELx 800™ BioTek®, USA; Microplate Strip Washer ELx 50™ BioTek®, USA) using commercial kits (Estradiol and Progesterone EIA Kit, Cayman Chemical Company, USA). The Coefficient of Variance of the intra- and inter-assay was 7.8% and 10.9 for progesterone, and 7.4% and 10.4% for estradiol.

Statistical analysis:

Pregnant buffaloes were categorized into two groups: group A (1 - <3 months pregnancy; n= 29) and group B (3 – 6 months pregnancy; n= 22). The Correlation Coefficients and *t* test were calculated using a commercial software programme, Statistica for windows, 1993.

RESULTS

Although there is no significant difference in the weight and diameter of corpora lutea between groups A (1 - <3 months pregnancy) and B (3 - 6 months pregnancy), the plasma and fecal progesterone concentrations are significantly ($p<0.05$) higher in group B than group A buffaloes (6.14 ± 0.61 Vs 4.09 ± 0.46 ng/ml and 2053.86 ± 232.28 Vs 1100.26 ± 230.03 ng/g, respectively; Table 1).

Table 2 shows a decrease in the number of follicles with all diameters in the group B buffaloes. This is coincided with the significant ($p<0.05$) decrease in plasma estradiol-17ß concentrations in group B (791.07 ± 75.59 pg/ml) in comparison to group A (1129.81 ± 137.47 pg/ml). There is a clear reduction in the number of all sized follicles in the group A and medium sized follicles in the group B with the presence of a corpus luteum (CL) on the same ovary (Table 2).

Table 1: Corpora lutea and progesterone concentrations (P_4) of pregnant buffaloes (mean ± SEM)

Parameters	n	Group A (1 – <3 months pregnancy)	n	Group B (3 – 6 months pregnancy)
Ovarian weight (g)	29	5.01 ± 0.38	22	5.01 ± 0.44
CL Ø (cm)	29	1.15 ± 0.07	22	1.20 ± 0.08
CL weight (g)	29	2.96 ± 0.13	22	3.26 ± 0.26
Plasma P_4 (ng/ml)	27	4.09^a ± 0.46	22	6.14^b ± 0.61
Fecal P_4 (ng/g)	19	1100.26^a ± 230.03	21	2053.86^b ± 232.28

Means with dissimilar superscripts in the same row are significantly different at $p<0.05$
Ø = diameter

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Table 2: Follicular activity (%) and estradiol-17ß concentrations (mean ± SEM) of pregnant buffaloes

Parameters	Group A (1 – <3 months pregnancy)			Group B (3 – 6 months pregnancy)		
	n	Ipsilateral with CL	Contralateral to CL	n	Ipsilateral with CL	Contralateral to CL
Follicles Ø <6 mm	26	11 (42.3%)	15 (57.7%)	20	10 (50%)	10 (50%)
Follicles Ø 6-10 mm	25	9 (36%)	16 (64%)	14	3 (21.4%)	11 (78.6%)
Follicles Ø >10 mm	15	4 (26.7%)	11 (73.3%)	3	2 (66.7%)	1 (33.3%)
Plasma estradiol (pg/ml)	27	$1129.81^{a} \pm 137.47$		20	$791.07^{b} \pm 75.59$	
Fecal estradiol (pg/g)	22	26509.38 ± 6373.90		18	25759.20 ± 9675.73	

Means with dissimilar superscripts in the same row are significantly different at $p<0.05$
Ø = diameter

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There are positive Correlation Coefficients ($p<0.05$; $r= 0.66$ and 0.85) between the ovarian weight and the weight of CL of pregnant buffaloes (Table 3). Similarly, positive Correlation Coefficients ($p<0.05$) present between the number of small follicles and plasma estradiol-17ß concentrations ($r= 0.97$), and between the number of large follicles and fecal estradiol-17ß concentrations ($r= 0.95$; Table 3). On the contrary, there are negative Correlation Coefficients ($p<0.05$) between the weight of corpora lutea and both plasma and fecal estradiol-17ß concentrations of buffaloes ($r= -0.79$ and $r= -0.86$, respectively; Table 3).

Table 3: Correlation Coefficients (r) between ovarian activity and hormonal concentrations of pregnant buffaloes (Group A, 1 – <3 months pregnancy)

Correlated parameters	n	Correlation Coefficients (r)
Weight of the left ovary X weight of the CL	13	r = 0.66*
Weight of the right ovary X weight of the CL	16	r = 0.85*
Number of follicles <6mm Ø X plasma estradiol level	18	r = 0.97*
Number of follicles >10mm Ø X fecal estradiol level	12	r = 0.95*
Weight of the right CL X plasma estradiol level	12	r = - 0.79*
Weight of the right CL X fecal estradiol level	12	r = - 0.86*

Ø = diameter

p<0.05

The effect of season on corpora lutea and progesterone concentrations of pregnant buffaloes is shown in Table 4. In group A, the ovarian weight and the diameter of CL decreased significantly ($p<0.05$) during Summer compared to Winter (4.68 ± 0.39 Vs 5.14 ± 0.49g and 0.94 ± 0.08 Vs 1.27 ± 0.07 cm, respectively). However, in group B, there is significant ($p<0.05$) increase in plasma progesterone concentrations during Summer in comparison to Winter (7.26 ± 0.99 Vs 5.01 ± 0.59 ng/ml, respectively; Table 4).

Table 5 shows that in group A, despite the presence of high number of the medium and large sized follicles during Winter, the plasma estradiol-17ß concentrations increased significantly ($p<0.05$) during Summer in proportion to Winter (1966.28 ± 234.98 Vs 777.62 ± 80.58 pg/ml).

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Table 4: Effect of season on corpora lutea and progesterone concentrations (P_4) of pregnant buffaloes (mean ± SEM)

Groups	Parameters	Winter (December – February)		Summer (May – July)	
		n	mean ± SEM	n	mean ± SEM
Group A (1 – <3 months pregnancy)	Ovarian weight (g)	19	$5.14^{a} \pm 0.49$	10	$4.68^{b} \pm 0.39$
	CL Ø (cm)	19	$1.27^{a} \pm 0.07$	10	$0.94^{b} \pm 0.08$
	CL weight (g)	19	2.95 ± 0.14	10	2.81 ± 0.30
	Plasma P_4 (ng/ml)	19	3.84 ± 0.54	8	4.70 ± 0.86
	Fecal P_4 (ng/g)	16	1126.56 ± 268.04	3	960.00 ± 361.35
Group B (3 – 6 months pregnancy)	Ovarian weight (g)	11	4.96 ± 0.49	11	5.06 ± 0.53
	CL Ø (cm)	11	1.34 ± 0.11	11	1.07 ± 0.12
	CL weight (g)	11	3.19 ± 0.46	11	3.16 ± 0.18
	Plasma P_4 (ng/ml)	11	$5.01^{a} \pm 0.59$	11	$7.26^{b} \pm 0.99$
	Fecal P_4 (ng/g)	10	2611.60 ± 816.91	11	1546.82 ± 694.09

Means with dissimilar superscripts in the same row are significantly different at $p<0.05$
Ø = diameter

Table 5: Effect of season on follicular activity (%) and estradiol-17ß concentrations (mean ± SEM) of pregnant buffaloes

Groups	Parameters	Winter (December – February)		Summer (May – July)	
		n	(%)	n	(%)
Group A (1 – <3 months pregnancy)	Follicles Ø <6 mm	7	(50.00 %)	7	(50.00 %)
	Follicles Ø 6-10 mm	13	(76.47 %)	4	(23.53 %)
	Follicles Ø >10 mm	11	(91.67 %)	1	(8.33 %)
	Plasma estradiol (pg/ml)	19	$777.62^{a} \pm 80.58$	8	$1966.28^{b} \pm 234.98$
	Fecal estradiol (pg/g)	16	25492.20 ± 7739.75	6	29221.87 ± 12016.52
Group B (3 – 6 months pregnancy)	Follicles Ø <6 mm	4	(30.77 %)	9	(69.23 %)
	Follicles Ø 6-10 mm	10	(76.92 %)	3	(23.08 %)
	Follicles Ø >10 mm	2	(66.67 %)	1	(33.33 %)
	Plasma estradiol (pg/ml)	9	741.97 ± 124.07	11	831.24 ± 96.45
	Fecal estradiol (pg/g)	7	21821.71 ± 10715.46	11	28264.87 ± 14662.13

Means with dissimilar superscripts in the same row are significantly different at $p<0.05$
Ø = diameter

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DISCUSSION

The mean weight and diameter of corpora lutea did not increase during the second trimester of pregnant buffaloes. Similar results were found in buffaloes (Hafez, 1955). Nevertheless, it was reported that the weight of CL increased slightly during the second period of gestation (75 – 132 days) in the Egyptian buffaloes (El-Sheikh *et al.*, 1969). Irrespective of persistent weight of corpora lutea during the second trimester of pregnancy, there was marked increase in progesterone concentrations in both peripheral plasma and feces during this period. The most plausible explanation for this increase in progesterone levels is the presence of additional sources of progesterone other than the CL such as placenta that secretes progesterone between days 150 – 250 of gestation in cattle (Thomas, 1997) and the adrenal glands that may contribute 1 – 4 ng/ml of progesterone during gestation (Wendorf *et al.*, 1983).

There were a decreased number of follicles with different diameters in the period of second trimester in pregnant buffaloes. This was accompanied with a significant decrease in plasma estradiol-17ß concentrations in this period of gestation. The same relationship between the size and number of follicles and estradiol-17ß concentrations was reported in cows (Brantmeier *et al.*, 1987). However, Robertson and King (1979) stated that the concentration of estrogens in the peripheral blood increased as pregnancy progress in cows. Ginther *et al.* (1996) observed a decrease in the dominant follicle diameter after day 90 of bovine pregnancy and attributed this to a decrease in luteinizing hormone (LH) pulse frequency and/or average LH concentrations, or to a poor number of LH receptors in follicular granulosa cells. Then, LH has a role in the growth and function of the largest follicles (Martin *et al.*, 2008). The dominant follicle is selected because it acquires LH receptors on its granulosa cells and that this allows the cells to synthesize estradiol in response to LH (Xu *et al.*, 1995; Fike *et al.*, 1997). Moreover, the periods of follicular growth not exceeding 6.0 mm in diameter observed during buffalo pregnancy could be consequence of an inadequate follicle stimulating hormone (FSH) support (Martin *et al.*, 2008).

There was a reduction in number of follicles with the presence of CL on the same ovary of pregnant buffaloes. In pregnant cows, corpora lutea on an ovary may modify the pattern of growth and atresia of follicles on the same ovary (Rexroad and Casida, 1975). They also stated that the corpora lutea may act on follicles to alter their growth rates to result in atresia at a smaller size or to increase their rate of growth to a large size at which they become atretic thus increasing their turnover rate. In addition, they claimed

that the possibility of this action is locally high concentrations of progesterone. High luteal progesterone levels have been shown to reduce the diameter of second dominant follicle in bovine (Bergfelt *et al.*, 1991; Fortune, 1993). Furthermore, progesterone has been shown to alter growth and atresia of follicles in rabbits (Wallach and Noreiga, 1970).

Positive Correlation Coefficients were estimated between the ovarian weight and the weight of CL of pregnant buffaloes. On the same direction, positive correlations between the weight of CL and ovary weight were reported in pregnant cows (Stormshak and Erb, 1961). Furthermore, in the current work, positive Correlation Coefficients were recorded between the number of follicles and plasma and fecal estradiol-17ß levels. Mellin and Erb (1965) found an estrogenic biological activity in peripheral blood and feces from pregnant cows. About 70% of total estrogen excreted in feces of cows (Monk *et al.*, 1975). However, in the present study, there were negative Correlation Coefficients between the weight of corpora lutea and both plasma and fecal estradiol-17ß concentrations. This may be attributed to the direct effect of corpora lutea on growth and atresia of follicles (Rexroad and Casida, 1975) through their high luteal progesterone levels (Bergfelt *et al.*, 1991; Fortune, 1993).

In the current study, the ovary was heavier and the diameter of corpora lutea was large in winter than in summer. The breeding efficiency of buffaloes is influenced by seasons (Yadava and Kushwaha, 1965; Rao and Pandey, 1982). Winter was proved to be the most favorable season for breeding of buffaloes (Yadava and Kushwaha, 1965; Roy *et al.*, 1972). Simultaneously, in cows, McNatty *et al.* (1984) reported that the mean diameter of large follicles was great and the corpora lutea were heavier in autumn and winter than in spring. The seasonal differences in ovarian activity are probably the consequence of seasonal differences in gonadotropin secretion (McNatty *et al.*, 1984), climatic factors and vegetative growth of crops (Yadava and Kushwaha, 1965).

The plasma progesterone levels in the second trimester of pregnant buffaloes were higher in summer than in winter. On the contrary, Rao and Pandey (1982) stated that there was low progesterone level in hotter months in comparison to cooler ones. Apart from the seasonal effect, explanation may be again be the presence of additional sources of progesterone in the second trimester of bovine pregnancy (Wendorf *et al.*, 1983; Thomas, 1997).

In the present study, the plasma estradiol-17ß concentrations during the first trimester were high in summer compared to winter. It is not clear whether this increase in plasma estradiol-17ß level is due to an imbalance in the pituitary gonadotropic complex as a result of neural stimuli from

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feto-placental unit or of some other factors such as stress leading to certain endocrine disturbances (Batra *et al.*, 1979; Rao and Pandey, 1982).

In conclusion, peripheral plasma and fecal progesterone concentrations increased and plasma estradiol-17ß levels decreased in the second trimester of pregnant buffaloes. Seasons exerted a significant effect on the ovarian activity during buffalo pregnancy.

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نشاط المبايض والعلاقة بين الهرمونات في الجاموس العشار

مجدي محمد وحيدالدين زكريا

قسم الدراسات الإكلينيكية – كلية الطب البيطري والثروة الحيوانية – جامعة الملك فيصل – المملكة العربية السعودية

أجريت هذه الدراسة على 51 جاموسة عشار بعد ذبحها حيث تم فحص الصفات الشكلية للمبايض وتقدير وزن وقطر الأجسام الصفراء وعدد وحجم الحويصلات. تم قياس معدل هرموني البروجستيرون والإستراديول-17بيتا في كلٍ من بلازما الدم والروث باستخدام تحليل الإنزيم المناعي.

أظهرت النتائج انخفاض عدد الحويصلات على المبايض مع تقدم الحمل. كذلك ارتفع معدل هرمون البروجستيرون وانخفض معدل هرمون الإستراديول-17 بيتا في كلٍ من بلازما الدم والروث كلما ازدادت فترة الحمل. كما وُجدت علاقة طردية بين وزن المبايض ووزن الأجسام الصفراء من جهة وبين عدد الحويصلات ومعدل هرمون الإستراديول-17 بيتا من جهة أخري. وقد تبيّن وجود علاقة عكسية بين وزن الأجسام الصفراء ومعدل هرمون الإستراديول-17 بيتا. كما أظهرت النتائج تأثيراً واضحاً للموسم على نشاط المبايض في الجاموس العشار تزامن مع التأثير القوي للموسم على معدل هرموني البروجستيرون والإستراديول-17 بيتا في بلازما الدم.

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