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ANATOMICAL AND HISTOLOGICAL CHANGES IN OVARIES OF DROMEDARY CAMEL (*Camelus dromedarius*) DURING PREGNANCY

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ABSTRACT

Genitalia including ovaries of slaughtered female camels at different stages of pregnancy were collected from slaughterhouses. They were examined to studying the morphological and histological changes of the ovary during different stages of pregnancy, early (1-3 mo), middle (4-8 mo) and late (9-12 mo).

Results revealed that average weight of the ovaries with corpora lutea (CLa) and of, the same ovaries without CLa did not differ significantly during the early (8.17 and 4.41 g) and middle stage (8.33 and 4.29 g). However, they significantly increased during the late stages of pregnancy (9.69 and 4.61 g). Weight and length of corpus luteum (CL) significantly increased only between the middle and late stages of pregnancy by about 7.4 and 3.7%, respectively, while diameter of CL significantly increased between the middle and late stage. Corpora lutea were located in the right ovaries in 43.3%, in the left ones in 50.0% and in both ovaries in 6.7% of all pregnancies. All embryos or foetuses were observed in the left uterine horns and frequent of ova or embryos migration from the right to the left side was 46.4%. Most of ovulation occurred in a single type in 86.7%, double type in 10.0% and triple in 3.3% of all pregnancies.

The histological examination of CL revealed that early signs of regression appeared at the end of the early stage of pregnancy (the 4th mo). This may indicate that the process of regression was slow and gradual without any changes in CL mass and somewhat changes in its shape.

Keywords: Camel, pregnancy, ovary, anatomy, histology.

INTRODUCTION

The ovary is considered as one of the main limitations of the reproductive performance of females. Great attention has been directed towards studying the morpho-histogenesis of non-pregnant ovary in camel during the follicular phase (Abdo *et al.*, 1969; Taher *et al.*, 1979 and El-Wishy and Hemeida, 1984) and luteal phase (Nawito *et al.*, 1979 and El-Wishy and Hemeida, 1984). However, few studies were carried out on the pregnant ovary in camel (El-Wishy *et al.*, 1981 and Ghoneim, 1985). Additional and detailed information on the ovary of camels are needed for further understanding the developmental changes occurring during different stages of the gestation period.

This study presents the morpho-histological development of the ovary in dromedary camel during the early, middle and late stages of the pregnancy with special references to corpus luteum.

MATERIALS AND METHODS

Pregnant genitalia including ovaries of 30 slaughtered female camels aged 4-9 years were collected from slaughterhouses. They were taken immediately to the Physiology Laboratory of the Animal Production Department, Faculty of Agricultural, El-Mansoura University. The mean age of the foetuses and then the stages of pregnancy were determined by estimating C.V.R.L. of foetuses for each genitalium (El-Wishy *et al.*, 1981). Nine animals were determined in each of the early (1-3 mo) and late (9-12 mo), however, twelve animals were in the middle (4-8 mo) stage of pregnancy.

Number of embryos or foetuses per each genitalium and number of corpora lutea (CLa) per each ovary were recorded. Weight of the ovaries carrying CLa was recorded, then CLa were separated at the border attached with the ovarian surface and were weighed. Length of the CLa was estimated as the distance between the attached and the free border of each corpus luteum (CL), however, the smallest and largest diameters were estimated in cross-sectional CL at its median part, then average diameter was calculated.

Specimens from ovaries carrying CLa and from CLa were fixed in neutral formalin for the histological examination. Section of 6-8 μm thickness were prepared and stained by haematoxylin and eosin (H&E) and Crossman's Trichrome stain (CTS) (Drury and Wallington, 1980). Statistical analysis was carried out according to Snedecor and Cochran (1980). The significant differences between stage means were tested by multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Macroscopical findings

Ovarian feature

Ovaries of the gravid genitalia examined at all pregnancy stages were characterized by presence of large well-developed CLa. This was in agreement with the findings of El-Wishy *et al.* (1981); El-Wishy and Hemeida (1984) and Ghoneim (1985). Majority of the CLa mass was prominently above the ovarian surface with central area of gray color in cross-sectioned CL (plate 1). El-Wishy (1992) described similar feature.

Various shapes of CLa were observed during the different stages of pregnancy. Spherical CLa during the early and late stages (plate 2) and oval CLa were found during the middle stage of pregnancy (plate 3). This confirms the previous reports of El-Wishy *et al.* (1981) and Ghoneim (1985).

At the early stage of pregnancy, the parts of CL attached to the ovarian surface were bluish or gray. While, the free poles were reddish-white in color and floppy in consistency changed to become bluish-white in color and firm in consistency during the middle and late stages of pregnancy. Ghoniem (1985) reported similar findings in dromedary camel.

Ovarian weight

During the early and middle stages of pregnancy, average weight of ovaries with CLa and of the same ovaries without CLa did not differ significantly, then increased ($P<0.05$) in weight during the late stage of pregnancy. On the other hand, average weight of the free ovaries did not

change significantly during all stages of pregnancy (Table 1). El-Wishy, et al. (1981) and Ghoneim (1985) reported similar trend of changes in weight of ovaries carrying CLa or free ovaries in dromedary camel.

Table 1: Changes in ovaries weight (g) during different stages of pregnancy.

Stage of pregnancy	Weight of ovaries carrying CLa (g)		Weight of the free ovaries (g)
	With CLa	Without CLa	
Early (1-3 mo)	8.17±0.07^{B}	4.41±0.08^{AB}	4.47±0.09
Middle (4-8 mo)	8.33±0.09^{B}	4.29±0.09^{B}	4.55±0.10
Late (9-12 mo)	9.69±0.06^{A}	4.61±0.09^{A}	4.41±0.09
Mean	8.40±0.07	4.43±0.06	4.47±0.07

A, B Values having different superscripts within the same column are significantly different at P<0.05.

The approximate similarity in mean weight of both ovaries without CLa and the free ovaries during the gestation period may indicate that most of changes in the ovarian weight were related mainly to changes in CL mass. In accordance with the present results, Shalash (1965) found that average weight of ovaries with CLa was 8.23 g and El-Wishy *et al.* (1981) reported a range of 6-11.33 g for ovaries with CL and of 2.5-6.4 g for the free ovaries in dromedary pregnant camel.

Corpora lutea measurements

Weight of CLa (Table 2) increased (P<0.05) by about 7.44% between the early and middle stage of pregnancy. This increase was associated with significant (P<0.01) increase by about 1.03% only in length of CLa. However, the late stage of pregnancy was characterized by marked increase in diameter of CLa without any changes in CLa mass. This may suggest two phases of changes in size of CLa during the pregnancy, the 1st phase longitudinal growth (oval CL) from the early stages to the middle stage of pregnancy (plate 3) and circular growth (spherical CL)up to the late stage of pregnancy (plate 2). Similar trend of changes in weight and size of CLa were reported by EL-Wishy and Hemeida (1984) and Ghoneim (1985).

Table 2: Changes in weight (g), length and diameter (cm) of CL during different stages of pregnancy.

Stage of pregnancy	Weight of CL (g)	Length of CL (cm)	Diameter of CL (cm)
Early (1-3 mo)	3.76±0.05^{B}	2.14±0.02^{B}	2.08±0.03^{B}
Middle (4-8 mo)	4.04±0.03^{A}	2.22±0.03^{A}	2.08±0.05^{B}
Late (9-12 mo)	4.08±0.08^{A}	2.30±0.04^{A}	2.28±0.05^{A}
Mean	3.96±0.04	2.22±0.04	2.15±0.03

A, B Values having different superscripts within the same column are significantly different at P<0.05.

Location of CL

Corpora lutea were located in the right ovaries in 43.3%, in the left ones in 50.0% and in both ovaries in 6.7% of all pregnancies (Table 3). These results are in agreement with El-Wishy (1988), who reported nearly similar values, being 44.9, 48.4 and 6.7%, respectively. Similar activity of

the right and left ovaries was reported by Ghoniem (1985) and El-Wishy (1992) in pregnant dromedary camel.

Table 3: Activity of the right and left ovary in pregnant camels.

Location of CL	Number of animals	%
On the right ovary	13	43.3
On the left ovary	15	50.0
On both ovaries	2	6.7
Total	30	100

Frequent of ova or embryos migration = [13 ÷ (13+15)] x 100=46.4% (Ghoniem, 1985)

The fairly equal activity of both ovaries in camel contrasted that reported in most of farm animals. Higher activity of the right ovary than the left one was stated in buffaloes (Usmani, 1992), sheep (Casida *et al.*, 1966) and goats (Taneja, 1959).

Site of pregnancy

Inspite of incidence of 43.3% of CLa on the right ovaries of pregnancies, examination of the gravid genitalia revealed that all embryos or foetuses were observed in the left uterine horns. This come in line with the results of El-Wishy and Hemieda (1984), who found a single foetus in the left horn of dromedary camel. However, Ghoneim (1985) indicated that 99.3% of pregnancies occurred in the left side in dromedary camel.

The high incidence of left horn pregnancy may be related to the differences in length of both uterine horns. Arthur and Al-Rahim (1982) mentioned that the right horns were always shorter than the left ones in camel. They added that the right horn could be supporting a conception up to 50 days of gestation. Several authors suggested that high incidence of left horn pregnancy may be related to frequent ova or embryos migration from the right to the left side. Frequent of ova or embryos migration calculated from the present results in table (3) was 46.4%. This is in agreement with a range of 30.0 to 48.8% reported by Shalash (1965) and Ismail (1987).

Frequency of multiple ovulation

Most of ovulation occurred in a single type (one CL/ovary) in 86.7%, followed by double ovulation (2 CL/ovaries/genitalium) in 10.0%, while triple ovulation (3 CL/ovaries/genitalium) occurred only in 3.3% of all pregnancies (Table 4). Musa and Abusineina (1976) and El-Wishy (1992) obtained similar results in camel.

Table 4: Frequency of multiple ovulation in dromedary camel.

Ovulation type	Number of pregnancies	Number of well developed CL	%
Single	26	26	86.7
Double	3	6	10.0
Triple	1	3	3.3
Total	30	35	100

Average number of CL/pregnancy = 35 ÷ 30 = 1.17
Ova wastage or embryonic loss = (35-30/35) x 100 = 14.3% (Ghoniem, 1985)

The ovulation in camel can be induced by coitus or cervical stimulation (Aria *et al.*, 1982), or by seminal plasma of male (Pan *et al.*, 1992). So, well-developed CLa are found on the ovaries surface only of the pregnant camel (Ismail, 1987). The present study indicated that the double and triple ovulation occurred only at the early stage of pregnancy. Although, multiple ovulation occurred in 13.3% of all pregnancies, a single foetus was found in each of gravid genitalium. This may be explained by a failure of fertilization of ova, wastage of fertilized ova or early embryonic death (El-Wishy *et al.*, 1981, Aria *et al.*, 1982 and Ghoniem, 1985).

In light of the present results, average number of CLa per each pregnancy was 1.17 and ova wastage or embryonic loss of about 14.3% of all ovulations (Table 4). Ghoneim (1985) reported similar results in dromedary camel.

Microscopical examination

At different stages of pregnancy, a single layer of flattened or cubical cells (Germinal epithelium, GE) covered the ovary (plate 4). The height of the covering epithelium ranged between 5-7 μm. Under beneath the epithelium, there was a dense fibrous connective tissue containing collegenous and reticular fibers (Tunica albuginea, TA) (plate 4).

At the early stage of pregnancy (1-3 mo), the cortical stroma was composed of spindle-shaped cells with central and dark stained nuclei, and contained blood capillaries (plate 4). Corpora albicantia (CA) and some ovarian follicles (OF) at early degrees of development and regression were detected to be embedded in the cortical stroma (Plate 5). During the middle stage of pregnancy (4-8 mo), different sizes of primary and atretic follicles were found in the cortical stroma. In the late pregnancy (9-12 mo) few primary follicles and some corpora albicantia were detected deeply in the cortical stroma, which contained more compact and polyhedral stromal cells.

Throughout the entire length of gestation period, the ovarian medulla was dense fibrous and tubular. Its stroma contained blood vessels and medullary tubules, which was lined with columnar or cubical epithelial cells (plate 6). It is worthy noting that ovarian structure did not differ markedly from that of non-pregnant ovary (Ghoneim, 1985).

The CLa of all pregnancies at different stages of pregnancy were covered with a well-developed dense irregular fibro-vascular connective tissue capsule. The capsule was mainly composed of parallel collagenous fibers and little of reticular fibers as well as stromal fibroblast cell and blood vessels (plate 7). Similar findings were obtained by Nawito *et al.* (1979) in normal CL of non-pregnancy camel and by Ghoniem (1985) in pregnant camel.

The surrounded capsule may be originated from tunica albuginea as a protective layer for CL tissue, its thickness was observed to increase by advancing the stage of pregnancy. Losing GE from the capsule of CL was observed and only TA was seen to cover all peripheral portions of CL.

Within the luteal tissue, septa of a thin connective tissue (Trabeculae) were seen to extend from the connective tissue of the capsule towards the central region of the CL forming central part of connective tissue and dividing the CL tissue into triangular lobules (Plate 8).

Plate 1: Cross section in CL mass showing central gray colored area.

Plate 2: Spherical CL during the early stage of pregnancy.

Plate 3: Oval CL during the middle stage of pregnancy.

Plate 4: GE, TA and cortical stroma (CS) at the early stage. (H&E) x 150

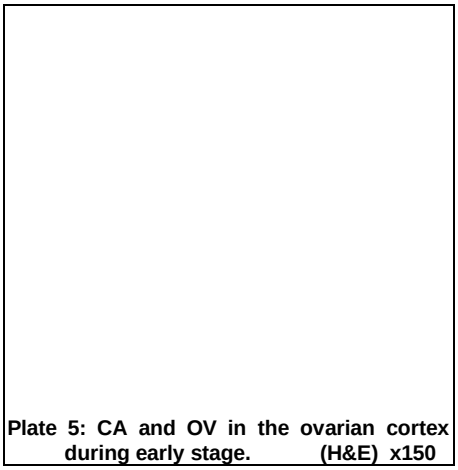

Plate 5: CA and OV in the ovarian cortex during early stage. (H&E) x150

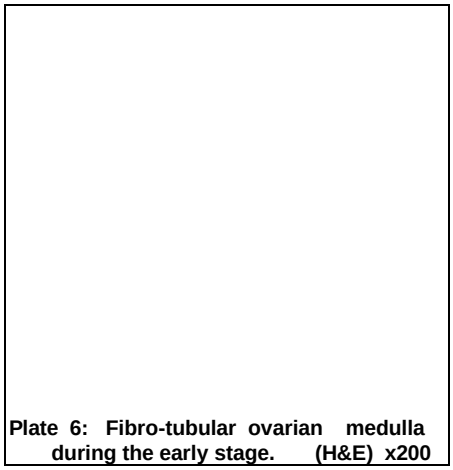

Plate 6: Fibro-tubular ovarian medulla during the early stage. (H&E) x200

At the early stage of pregnancy, the luteal tissue was composed of two basic types of cells surrounded by reticular fibers. The first large cells were similar to the granulosa cells and may be derived from membrana granulosa of the ovulated follicle (lutien granular cells). These cells were usually associated with smaller ones. They had light stained and granular cytoplasm, and large vesicular nuclei. The second type of cells were similar to the theca interna cells (lutein theca cells). They were small-sized and irregular in shape with intensively stained cytoplasm and eccentricity large oval nuclei. The thecal cells were always located in between the granular cells on the blood capillaries (Plate 8).

Early degeneration in large luteal cells was noted at the end of the early stage (3 mo) and at beginning of the middle stage of pregnancy (4 mo). Some vacuoles tended to appear in cytoplasm of the granular cells (in 90% of CLa) scattered at borders of CL and also tendency of decrease in size of the luteal cells. The nuclei were seen to folded and darkly stained by eosin (Plate 9).

At the end of the middle stage and during late stage of pregnancy, in about 92% of CLa large detectable spaces were noted between the lutein cells and stromal cells began to increase between the lutein cells and were accumulate in the center of CL (Plate 10). At the end of the late stage marked decrease in number and size of the luteal cells (Plate 11). In some CLa, these spaces were replaced by intercellular stroma were noted in all CLa of pregnancy (Plate 12).

The current study suggested that signs of regression of CL appeared at the end of the early stage of pregnancy (the 4^{th} mo). The process of regression was slow and gradual with maintained CL mass. Signs of regression could be detected microscopically without any changes in the morphological measurements of CL (Table 2). Ghoneim (1985) reported that signs of regression of CL of pregnant camel were noted as early as 82 days of gestation.

Concerning the types of regression in camel, Abdel-Razik (1979) stated that regression of CL was in two ways vascular and avascular and

Ghoneim (1985) described slow and rapid regression. However, in sheep, Dean *et al.* (1966) found that degeneration of the nuclei was the first sign of regression followed by vacuolation. Yet, in buffaloes the CL regression was gradual and associated with vacuolar degeneration (Mustafa *et al.*, 1988).

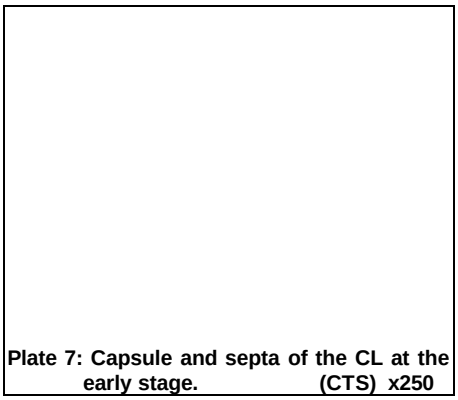

Plate 7: Capsule and septa of the CL at the early stage. (CTS) x250

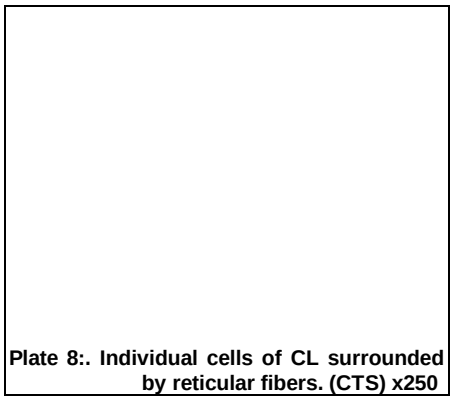

Plate 8:. Individual cells of CL surrounded by reticular fibers. (CTS) x250

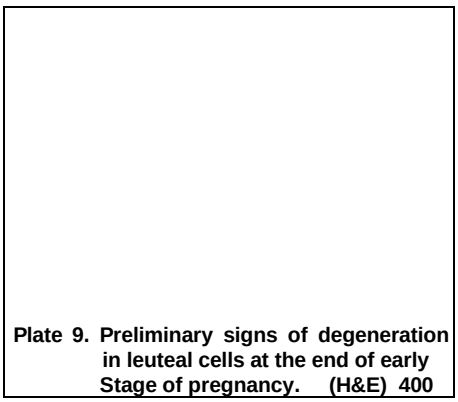

Plate 9. Preliminary signs of degeneration in leuteal cells at the end of early Stage of pregnancy. (H&E) 400

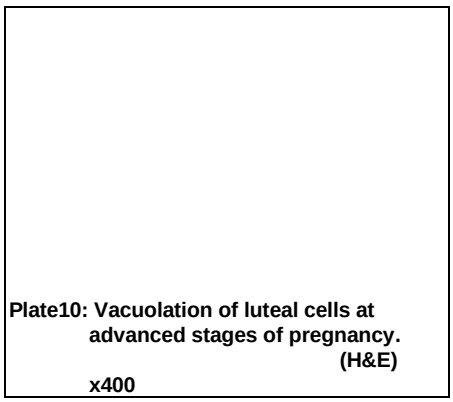

Plate10: Vacuolation of luteal cells at advanced stages of pregnancy. (H&E) x400

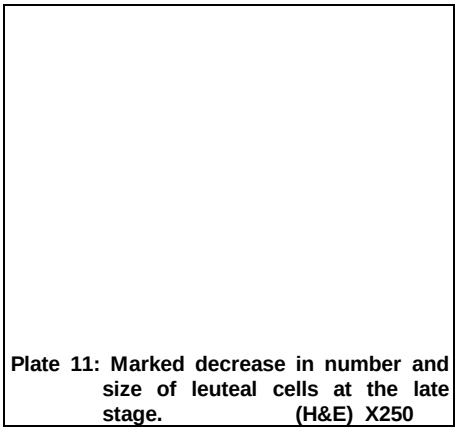

Plate 11: Marked decrease in number and size of leuteal cells at the late stage. (H&E) X250

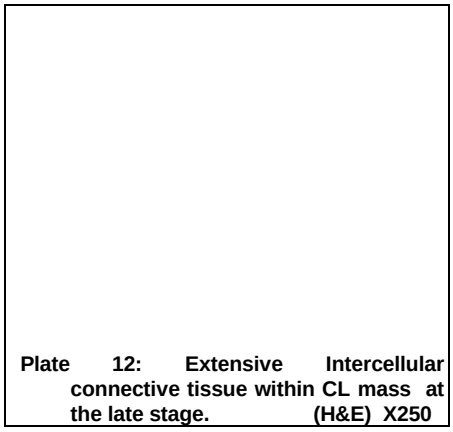

Plate 12: Extensive Intercellular connective tissue within CL mass at the late stage. (H&E) X250

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التغيرات التشريحية والهستولوجية في مبايض الجمل وحيد السنام أثناء الحمل

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استخدم في هذه الدراسة القناة التناسلية شاملة المبايض للجمال المذبوحة على مراحل مختلفة من فترة الحمل والتي تم تجميعها من المجازر وذلك لدراسة التغيرات التشريحية والهستولوجية في مبيض الجمال أثناء فترة الحمل. وقد أوضحت النتائج الآتي:

1- وجود جسم أصفر في المراحل المختلفة من الحمل والذي اختلف شكله من كروى أثناء المرحلة المبكرة (1-3 شهور) والمتأخرة (9-12 شهر) من الحمل وبيضاوي أثناء المرحلة المتوسطة من الحمل (4-8 شهور).
2- لم يختلف متوسط وزن المبايض التي تحمل جسم أصفر أو لنفس المبايض بعد إزالة الجسم الأصفر معنوياً أثناء المراحل المبكرة والمتوسطة من الحمل ، بينما زاد متوسط الوزن لكل منهما معنوياً في المرحلة المتأخرة من الحمل.
3- زاد متوسط وزن وطول الجسم الأصفر معنوياً بين المراحل المتوسطة والمتأخرة من الحمل بحوالي 7,4% ، 3.7% على الترتيب ، بينما زاد متوسط قطر الجسم الأصفر معنوياً بين المراحل المتوسطة والمتأخرة من الحمل.
4- كانت الأجسام الصفراء موجودة على المبايض اليمنى موجودة في 43.3% من الجمال العشر وعلى المبايض اليسرى في 50% وعلى كل من اليسرى واليمنى في 6.7%.
5- جميع الأجنة كانت موجودة في القرن الأيسر بنسبة 100% ومعدل هجرة البويضات أو الأجنة من القرن الأيمن إلى الأيسر حوالي 46.4%.
6- معظم عمليات التبويض حدثت في شكل فردى (جسم أصفر واحد/مبيض) بنسبة 86.7% وفى شكل زوجي (2 جسم أصفر/مبيض) بنسبة 10% وفى شكل ثلاثي بنسبة 3,3% من النوق العشار.
7- أثبت الفحص الهستولوجى أن هناك علامات مبكرة لاضمحلال الجسم الأصفر تبدأ عند نهاية المراحل المبكرة من الحمل (نهاية الشهر الثالث أو بداية الشهر الرابع) ، وتزيد هذه العلامات في المرحلة المتأخرة من الحمل.

وتستنتج الدراسة أن عملية الاضمحلال في التركيب النسيجي للجسم الأصفر في الجمال قد تكون بطيئة وتدريجية مع تغير الشكل بدون التأثير على كتلة الجسم الأصفر وهى التي تختلف عما يحدث في الحيوانات الأخرى.