

SOME REPRODUCTIVE PARAMETERS OF NON-ADULT, ADULT NON-PREGNANT AND PREGNANT SHE CAMEL SLAUGHTERED IN ASSIUT GOVERNORATE

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SUMMARY

The aims of this study were to investigate the anatomy and ovarian activity of camel reproductive tract in addition to determination of glucose and urea concentrations in both serum and follicular fluid. Female reproductive tract (n=20) and blood samples were collected from the slaughtered she camels. Based on the ovarian structures, the reproductive tracts of she camels were classified into non-adult (no corpora lutea or albicantia on the ovaries), adult non-pregnant (corpora albicantia and/or corpora lutea < 4 g on the ovaries) and pregnant (corpora albicantia and corpora lutea > 4 g on the ovaries). Blood samples were collected during slaughter process from each animal. Weights of ovaries and corpora lutea were recorded as well as numbers of follicles, corpora lutea and albicantia. The follicles were classified according to their diameter to small (<0.3 cm) medium (0.3-0.8 cm) and large (> 0.8 cm). Medium and large follicles were aspirated to collect follicular fluids and oocytes. Quality of oocytes was evaluated after classification into cumulus enclosed; partially cumulus enclosed and denuded oocytes. Weight and measurements of reproductive tracts were determined.

The results indicated that ovaries of slaughtered she camels enclosed in an ovarian bursa, had flattened shape, and follicles giving the ovaries the appearance of a bunch of grapes. Weights of ovaries were less ($P<0.05$) in non-adult animals compared to adult non-pregnant and pregnant ones. Corpora lutea and/or albicantia were found on the right and left ovaries of the adult non-pregnant and/or pregnant animals. She camel has a bicornuate uterus, where the left horn was longer ($P<0.05$) than the corresponding right one in non-adult, adult non-pregnant and pregnant animals. The length of cervix was 1.9 ± 0.1 , 3.2 ± 0.4 & 3.7 ± 0.5 cm with 3 to 4 rows of outgrowth ridges in non-adult, adult non-pregnant and pregnant animals, respectively. The length of vagina was 4.2 ± 0.76 , 21.0 ± 5.3 & 26.6 ± 2.7 cm in non-adult, adult non-pregnant and pregnant animals, respectively. Majority of collected oocytes were cumulus enclosed (66.7%) whereas the remaining ones were partially cumulus enclosed (25.0%) and denuded (8.3%). Urea concentration in serum (29.3 ± 6.1) was reflected in the follicular fluid (31.3 ± 13.4).

Keyword: Camel, reproductive tract, ovarian structure and measurement

INTRODUCTION

Camels are classified as dual purpose in addition to be race animals (Wardeh, 2004). Camels (*Camelus dromedarius*) contribute in producing milk and meat under pastoral systems in the Afro-Asian dry land belt (Kaufman, 2005). Average of produced milk and meat varies according to species (Wardeh, 2004). Moreover,

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camels are adopted to produce under scarcity of water, poor quality feed and severe heat stress (El-Wishy, 1988).

Camel milk might play a protective role against tissue damage mediated by free radicals (Al-Humaid *et al.*, 2010). However, the reproductive potentiality should be improved. To exploit the recent techniques (e.g. superovulation, embryo transfer and cloning of camel (Skidmore *et al.*, 1998 and Wani *et al.*, 2010) understanding of the reproductive biology of camels including anatomy of the reproductive tract is required.

Ali *et al.* (2007) determined serum and follicular fluid concentrations of some biochemical metabolites (glucose, cholesterol, total protein, albumin, globulin and triglycerides) during the low and the peak breeding seasons in she camels in relation to the follicular size. They found no relation between serum and follicular fluid contents of the aforementioned metabolites. Wani and Skidmore (2010) reported that biochemical changes in serum and follicular fluid influenced reproductive outcome. In the light of the previous studies, decrease in serum glucose concentration (Yan *et al.*, 2008) in mice and increase serum urea concentration in sheep (Mohammed *et al.*, 2011) were adversely affected follicle numbers and sizes and oocytes' quality. Iwata *et al.* (2006) concluded that blood urea nitrogen is a predictable index of the developmental competence of bovine oocytes. Furthermore, Mohammed and Attaai., (2011) found that elevated serum urea retarded timing of embryo cleavage at earlier stages in mice.

The aim of the present study was to characterize the reproductive tracts and ovarian activity of she camel; to find out the relation between follicular fluid and serum contents of urea and glucose concentrations and to evaluate the quality of collected oocytes.

MATERIALS AND METHODS

Collection of reproductive tracts of female dromedary camels:

Twenty reproductive tracts of female dromedary camels slaughtered in Assiut governorate were collected during October and November (peak of breeding season). Based on corpora lutea or corpora albicantia observed on the ovaries, the genital tract were classified into non-adult (no corpora structures), adult non-pregnant (corpora albicantia and/or corpora lutea < 4 g in the ovaries) and pregnant (corpora albicantia and corpora lutea > 4 g in the ovaries). The collected genital tracts were kept in a cool box (30-33°C) and transported to the laboratory within 4 hours.

Collection of blood samples:

Blood sample (about 10 ml) was collected from all experimental she camels and centrifuged at 400 rpm for 15 minutes for serum separation. Serum was decanted into Eppendrofe tubes and stored at -20 °C until the analysis of glucose and urea.

Measurements of reproductive system:

Weights and length of reproductive tract were recorded. Weight and numbers of follicles on each ovary were recorded as well. The follicles were classified into small (< 0.3 cm); medium (0.3-0.8 cm) and large (> 0.8 cm) to evaluate the oocytes, which expected to be developed *in vitro*. Follicles (≥ 0.3 cm) were aspirated for collection of follicular fluid and oocytes using syringe with a needle of 18-gauge. The collected follicular fluid was stored in Eppendrofe tubes at -20°C for the analysis of glucose

and urea. Numbers and quality of the collected oocytes were recorded in Ringer's medium supplemented with 10% serum. Oocytes were categorized into three classes based on the cumulus cells and homogeneity of the cytoplasm (Tornera *et al.*, 2003) as:

Grade 1 (cumulus-enclosed): Oocytes were completely invested with cumulus cell layers (good oocytes).

Grade 2 (partial-enclosed): Oocytes were surrounded with scanty cumulus cell layers (fair oocytes)

Grade 3 (denuded oocytes): Naked (denuded) oocytes.

Determination of serum glucose and urea concentrations:

Urea and glucose in both serum and follicular fluid were determined calorimetrically using readymade commercial kits (diamond kit, Egypt) according to the method described by the manufacturer.

Statistical analysis:

Data are presented as means \pm SD. Differences between mean values were determined by ANOVA procedures of SAS (1998) followed by comparisons using the Duncan's multiple range test. Differences with $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Out of twenty reproductive tracts examined, three were for non-adult (15%), 10 for adult non-pregnant (50%) and the rest seven were for pregnant animals (35%).

Reproductive tract measurements of non-adult, non-pregnant and pregnant camels:

Weight of reproductive tracts increased ($P < 0.05$) with age advancement, from non-adult to adult irrespective of pregnancy, which agree with the findings of Mohammed (2009). Moreover, in pregnancy cases, weight and size of reproductive tract were consequently increased (Plate 1, a-c).

Uterus had bicornuate shape like the letter Y (Plate 1 a-c). The present findings agree with the findings of Arthur *et al.* (1986), while disagree with the findings of Srikandakumar *et al.* (2001), who described camel uterus as bipartite type. Uterus length and diameter increased ($P < 0.05$) from non-adult, non-pregnant and pregnant camels (Plate 1). Unlike ruminants, the left parts of the reproductive tract of she-camel (oviduct and uterine horn) were longer than the right parts in all ages (Plate 1, a-c). This finding comes close to the findings of Chen and Yuen (1984); Arthur *et al.* (1986); and Srikandakumar *et al.* (2001). Arthur *et al.* (1986) also reported that the left uterine horn is distinctly larger than the right, even in the fetus. The diameter of the left uterine horn was slightly more than the right one and increased during pregnancy, with short uterine body (Table 1).

Two fetuses were collected from the left horn of uterus whereas the two corpora lutea (CLs), were detected on the right ovary, which indicated possibility of redistribution of embryos during pregnancy in cases of twines. (Plate 1 i). In another case, placenta was collected from the left horn with an area of 900 cm² (length x width 50 x 18.0 cm, respectively) (Plate 1 j). In the present study, the fetus was solely exists in the left uterine horn which agree with the results of ElWishy (1988),

Srikandakumar *et al.* (2001) reporting pregnancy in she-camels is usually occurred in left horn and unlike other ruminants, where implantation can occur in either horns.

Table 1. Characteristics of reproductive tract of non-adult, non-pregnant and pregnant camels

Items	Slaughtered animal		
	Non-adult	Non-pregnant	Pregnant
Physiological status			
Number of reproductive system	3	10	7
Weight of reproductive system, g	62.1 ^b ± 12.5	434.0 ^a ± 164.6	595.7 ^a ± 110.9
Length of right oviduct, cm	11.0 ^b ± 1.0	18.5 ^a ± 5.3	20.7 ^a ± 5.5
Length of left oviduct, cm	13.0 ^b ± 1.0	23.7 ^a ± 5.5	24.3 ^a ± 6.4
Length of right horn, cm	4.2 ± 0.76	6.2 ± 0.9	11.6 ± 9.2
Diameter of right horn in the middle, cm	2.1 ^b ± 0.4	2.7 ^{ab} ± 0.3	5.5 ^a ± 3.8
Length of left horn, cm	5.7 ^b ± 1.32	10.7 ^{ab} ± 2.8	17.7 ^a ± 5.0
Diameter of left horn in the middle, cm	2.1 ^b ± 0.3	3.6 ^b ± 0.7	9.4 ^a ± 2.3
Length of uterine body, cm	2.1 ^b ± 0.06	5.9 ^a ± 1.1	6.6 ^a ± 1.8
Length of cervix, cm	1.9 ^b ± 0.1	3.2 ^a ± 0.4	3.7 ^a ± 0.5
Length of vagina, cm	4.2 ^c ± 0.76	21.0 ^b ± 5.3	26.6 ^a ± 2.7

Values are presented as means ± standard deviation

a,b,c: Values with the different superscripts on the same row differ at P<0.05

Ovarian measurements of non-adult, non-pregnant and pregnant camels:

The appearance and size of ovaries vary according to the age and activity of the animals (Table 2). In the non-adult animals they have a smooth surface with visible vesicles (< 3 cm in diameter) throughout the surface which correspond to the follicles (Plate 1, d). In non-pregnant females the ovaries are oval or circular, flattened laterally and have an irregular surface due to many small follicles. Large follicles and current corpora albicantia project from the main contour of the ovary and give it a more lobular form (Plate 1, e). This lobulation increases with increased number of previous ovulations or pregnancies and is mainly due to the presence of old corpora albicantia.

Weight of ovaries were increased (P<0.05) upon ovulation and formation of corpus luteum (Plate 1, d-g). Weight of ovaries and CLs in pregnant camel was heavier than non-adult and adult ones. In pregnant camel, the right ovary is heavier than the left ovary due to the high number of CLs (1.6 ± 0.54) than the left one (1.0 ± 0.0) (Table 2). In the seven pregnant she camels, corpora lutea were found in both right and left ovaries. In case of two CLs were found on the same ovary, one of them was larger than the other one (Plate1, f). Corpora lutea of the collected reproductive tracts of pregnant animals had a weights of 4.5 – 6.7 g. Furthermore, the right (2.14 vs. 1.6) and left (2.85 vs. 0.9) ovaries of pregnant animals, respectively, contained more corpora albicantia than those of non pregnant animals (Table 2 and Plate1, d-f). In the non-pregnant camel, Osman (1965) and ElWishy (1992) found that the CL measures 12 - 15 mm in diameter and weighs 1.5 - 2 g but during pregnancy the size and weight increases to an average of 22 ± 6 mm and 4.9 ± 1 g respectively.

Ovarian activity of non-adult, non-pregnant and pregnant camels:

The range numbers of visible vesicular follicles seen on the ovaries of non-adult, non-pregnant and pregnant camels in the present study were 2-5, 10-77 and 44-50 respectively. Although the follicles with diameter 0.3 - 0.8 and > 0.8 cm were not seen on the ovaries of non-adult camel, they were (4.1 & 0.4) and (3.0 & 0.4) respectively of non-pregnant and pregnant camels (Table 2). In other studies (Skidmore *et al.*, 1996) the ovarian follicular wave pattern and follicular activity is

dominated by 4 types of follicles, namely: small growing follicles, mature follicles, regressing follicles or over-large, anovulatory follicles. As the follicular waves overlap with each other, several generations of follicles may be present at the same time (Skidmore *et al.*, 1996). The small growing follicles are visible on the surface of the ovary as small slightly raised vesicles measuring between 2 - 4 mm, whereas the mature pre-ovulatory follicle measures between 13 – 20 mm (Tibary and Anouassi 1996; Skidmore *et al.*, 1996) and is spherical, turgid, with a thin clear translucent wall and protrudes markedly from the ovarian surface (Plate 1 h).

The appearance of regressing follicles depends on the stage of regression. At the start of regression the follicular wall becomes thick and opaque and the diameter decreases slowly until the follicle recedes into the ovary itself. Large anovulatory follicles are present in about 50% of non-mated females and their size and appearance was highly variable (Tibary and Anouassi 1996; Skidmore *et al.*, 1996). They vary in size from 25 - 60 mm and may have a thin or thick, opaque wall and contain either serous or haemorrhagic fluid with various amounts of fibrin.

Table 2. Ovarian measurements and activity of non-adult, non-pregnant and pregnant camels

Item	Slaughtered animal		
	Non-adult	Non-pregnant	Pregnant
Physiological status			
Weight of right ovary, g	0.93 ^c ±0.25	4.04 ^b ±1.13	7.46 ^a ±2.8
Weight of left ovary, g	1.09 ^b ±0.24	4.2 ^a ±1.1	4.78 ^a ±2.5
Size of right ovary, cm	1.3 ^b x 1.1 ^b x 0.4 ^b	3.02 ^a x 2.0 ^a x 0.7 ^a	2.9 ^a x 2.2 ^a x 0.9 ^a
Size of left ovary, cm	1.4 ^b x 1.1 ^b x 0.4 ^b	3.6 ^a x 2.3 ^a x 0.8 ^a	3.2 ^a x 2.2 ^a x 0.8 ^a
Follicles on the right ovary, <0.3 cm	2.6±0.6	22.8±10.7	25.6±21.1
Follicles on the right ovary, 0.3-0.8 cm	0.0±0.0	4.8±3.22	4.3±2.7
Follicles on the right ovary, >0.8 cm	0.0±0.0	0.1±0.3	0.0±0.0
Follicles on the left ovary, <0.3 cm	3.0±1.7	35.6±26.6	26.9±18.4
Follicles on the left ovary, 0.3-0.8 cm	0.0±0.0	4.1±3.44	3.0±2.4
Follicles on the left ovary, >0.8 cm	0.0±0.0	0.4±0.7	0.4±0.8
Number of CL on the right ovary	0.0 ^b ±0.0	0.0 ^b ±0.0	1.6 ^a ±0.5
Weight of CL on the right ovary	0.0 ^b ±0.0	0.0 ^b ±0.0	5.5 ^a ±1.0
Number of CL on the left ovary	0.0 ^b ±0.0	0.1 ^b ±0.0	1.0 ^a ±0.0
Weight of CL on the left ovary	0.0±0.0	2.1±0.0	4.6±0.1
Number of C. Alb. on the right ovary	0.0 ^b ±0.0	1.6 ^a ±1.17	2.14 ^a ±1.07
Number of C. Alb. on the left ovary	0.0 ^b ±0.0	0.9 ^b ±0.7	2.8 ^a ±1.77

Values are presented as means ± standard deviation

a,b,c: Values with the different superscripts on the same row differ at P<0.05

CL: Corpus luteum; C. Alb.: corpora albicantia

Morphology of collected oocytes :

A total of 35 follicles of diameter ≥0.3 cm were aspirated to collect oocytes from non-pregnant and pregnant she camels. Twenty four oocytes were collected with efficiency of 68.6% (24/35). Majority of the collected oocytes were of grade 1 (66.7, 16/24), whereas the remaining oocytes were either grade 2 (25%, 6/24) or grade 3 (8.3, 2/24) (Fig 1).

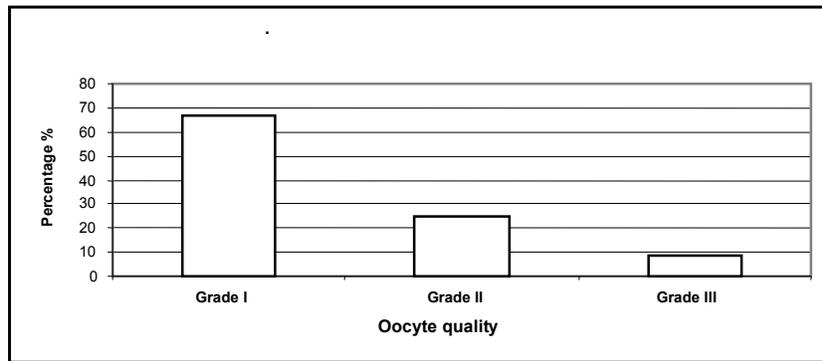


Figure 1: Quality of collected oocytes from the slaughtered she-camels

Tornera *et al.* (2003) isolated oocytes from sliced ovaries of she camel after slaughtering of non-pregnant and pregnant donors. The morphology of oocytes was divided into COCs with compact (26.9 and 28%), dispersed (39.3 and 46%), corona radiata cumulus investment (27.9 and 21.7%) and without cumulus (6 and 4.2%), respectively for pregnant and non-pregnant donors. The COCs of non-pregnant and pregnant donors with compact and dispersed cumulus cells were collected together in this study (66.7%), which is lower than that reported in the previous studies. This might be related to increase or decrease metabolites concentrations such as glucose and urea in serum and follicular fluid. Davoodian *et al.* (2010) conducted a study to describe in detail the ultrastructural features and morphological characteristics of camel oocytes from preantral follicles in relation to the sequential stages of follicular development and also for oocytes from antral follicles in relation to their diameter. They found that the growth of camel oocyte is associated with progressive increase in the number of granulosa cells, mitochondria, endoplasmic reticulum, Golgi complexes and cytoplasmic vesicles as well as decrease in the number of lipid droplets and the nucleus migration from an eccentric in preantral to a peripheral location in antral follicles.

Urea and glucose concentrations:

Urea and glucose concentrations in serum and follicular fluid are presented in Table (3) indicating no significant difference in the concentrations of follicular fluids and blood serum. Results of urea concentrations in serum of non-pregnant animals are in agreements with those obtained by Ahmed and Omer (2009). They found that urea and glucose concentrations respectively in camel serum were 28.7 ± 1.42 and 162.16 ± 18.8 mg/100ml. This might be explained the low quality of the obtained oocytes in this study. Decrease serum glucose concentration (Yan *et al.*, 2008) was adversely affected follicle number and size as well as oocyte quality. In addition, the adverse effect of urea on the oocyte is likely to involve inhibition in the growth and metabolism of the oocyte-supporting granulosa cells (Rooke *et al.*, 2004). Zia-Ur-Rahman *et al.* (2008) investigated ovarian follicular fluid and serum biochemical, hormonal, electrolytes and amino acids profiles in female dromedary camel (*Camelus dromedarius*). This study is indicative of either low or high concentrations of certain biochemical metabolites, hormones, electrolytes and amino acids in small and large



Plate 1. Illustrate the reproductive systems of non-adult, non-pregnant and pregnant she camels

Table 3. Urea and glucose concentrations (mg/dl) in serum and follicular fluid

Items	Serum	Follicular fluid
Urea	29.3 ± 6.1	31.3 ± 13.4
Glucose	66.2 ± 4.0	70.7 ± 6.9

follicles for the individual roles that they play in the growth and development of follicles in the one-humped she-camel

CONCLUSION

It could be concluded that follicles are found in the slaughtered she-camels, non pregnant and pregnant. Therefore, ovum-pick up of COCs can be applied of life non pregnant and pregnant she-camels. The collected COCs could be used for *in vitro* production of embryos (IVP). Transfer of blastocysts in she-camel should be done to the left side of uterus. Studies of *in vitro* production of embryo, freezing and vitrification of embryos are still required.

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بعض القياسات التناسلية لإناث الجمال غير البالغة وبالغة غير العشار والعشار المذبوحة بمحافظة أسيوط

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الهدف من هذه الدراسة هو تقدير بعض القياسات للقناة التناسلية ونشاط المبيض لإناث الجمال المذبوحة بمحافظة أسيوط بالإضافة الي تركيزات اليوريا والجلوكوز في كلا من السيرم والسائل الحويصلي. تم جمع الأجهزة التناسلية وعينات الدم للحيوانات المذبوحة. بناءا علي تركيب المبيض ، قسمت الأجهزة التناسلية المجموعة الي أجهزة من حيوانات صغيرة غير بالغة (لا توجد أجسام بيضاء أو صفراء علي المبايض) ، أجهزة من حيوانات بالغة غير حامل (توجد أجسام بيضاء و/أو صفراء علي المبايض وزنها اقل من ٤ جم) ، أجهزة من حيوانات حامل (توجد أجسام بيضاء وصفراء علي المبايض وزنها اكبر من ٤ جم). جمعت عينات الدم أثناء عملية الذبح من كل حيوان. تم تقدير وزن المبايض والأجسام الصفراء وكذلك عدد الحويصلات والأجسام الصفراء والبيضاء. قسمت الحويصلات بناءا علي حجمها الي صغيرة (قطرها اقل من ٣مم) ومتوسطة (قطرها ٣-٨مم) وكبيرة (قطرها اكبر من ٨مم). سحبت محتويات الحويصلات المتوسطة والكبيرة لجمع السائل الحويصلي والبويضات. قسمت البويضات على حسب درجة جودتها إلي بويضات محاطة بخلايا الركام ، بويضات محاطة جزئيا بخلايا الركام ، بويضات غير محاطة بخلايا الركام. تم وزن وقياس أجزاء القناة التناسلية. أوضحت النتائج أن المبايض كانت مسطحة ومحتوية علي عدد كبير من الحويصلات علي شكل عنقود العنقب. كل مبيض محاط بالكيس المبيضي. كانت أوزان المبايض اقل في الحيوانات الغير بالغة ($P < 0.05$) مقارنة بالحيوانات البالغة غير العشار والبالغة العشار. ولقد وجدت الأجسام الصفراء والبيضاء في كلا المبيضين الأيسر والأيمن في الحيوانات البالغة غير العشار والعشار. الجمال لها رحم من نوع ال Bicornuate. قرن الرحم الأيسر أطول ($P < 0.05$) من قرن الرحم الأيمن في الحيوانات الغير بالغة والبالغة غير العشار والعشار. طول عنق الرحم كان 1.9 ± 0.1 ، 3.2 ± 0.4 ، 3.7 ± 0.5 سم في الحيوانات الغير بالغة والبالغة غير العشار والعشار علي الترتيب ومحتويا علي ٣-٤ حلقات من النموات الحاجزة. طول المهبل كان 4.2 ± 0.76 ، 5.3 ± 0.3 ، 26.6 ± 2.7 سم في الحيوانات الغير بالغة والبالغة غير العشار والعشار علي الترتيب. معظم البويضات المجموعة كانت محاطة بخلايا الركام (٦٦.٧%) في حين أن نسبة البويضات الباقية كانت محاطة جزئيا بخلايا الركام (٢٥.٠%) وغير محاطة (٨.٣%). تركيزات اليوريا في السيرم (29.3 ± 6.1) انعكست في السائل الحويصلي (31.3 ± 13.4).