

***In Vitro* Maturation of Camel Oocytes As Affected By Different Media during Breeding and Non-Breeding Seasons**

A.E.B. Zeidan¹, M.A. El-Harairy², Sh.A. Gabr³, M.A. Tag El-Dien¹, S. A. Abd El-Rahman⁴ and A.M. Amer¹

¹Animal Production Research Institute, Dokki, Giza, Egypt.

²Department of Animal Production, Faculty of Agriculture, Mansoura University, Egypt.

³Department of Animal Production, Faculty of Agriculture, Tanta University, Egypt.

⁴Biology Department, Faculty of Science, Al-Mostansiriya University, Iraq.

Abstract: A total number of 220 clinically healthy she-camel was used in this study. The age of these camels varied from 5 to 10 years and their weights were approximately 500-600 kg. Two experiments were carried out. The first experiment aimed to define the effect of different seasons of the year on follicular fluid components and ovarian activity either in the right or left ovary. The second experiment designed to define the effects of various maturation media (TCM 199, Ham's F-10, Basal and Hank's) on the *in vitro* maturation of camel oocytes during breeding and non-breeding seasons. In the first experiment, the obtained results showed that ovary weight and number of corpora lutea were significantly ($P < 0.05$) higher during spring, winter and autumn seasons, than summer season. Numbers of the normal follicles were significantly ($P < 0.05$) higher during spring, while the atretic follicles were significantly ($P < 0.05$) higher during summer season than other seasons. Oocytes recovery, compact oocytes complexes (COC's) and partially denuded cumulus oocytes (PDCO) were significantly ($P < 0.05$) higher during autumn, while expanded cumulus oocytes (ECO) and denuded cumulus oocytes (DCO) were significantly ($P < 0.05$) higher during spring and winter seasons than other seasons of the year. The highest ($P < 0.05$) activities of follicular fluid aspartate – aminotransaminase (AST), alanine – aminotransaminase (ALT), alkaline phosphatase (ALP) and acid phosphatase (ACP) enzymes were recorded during summer and the lowest ($P < 0.05$) activity was recorded during spring season. The highest ($P < 0.05$) values of follicular fluid potassium and calcium were recorded during winter and the lowest ($P < 0.05$) values were recorded during summer season. Testosterone concentration was significantly ($P < 0.05$) higher, however cholesterol concentration was significantly ($P < 0.05$) lower during summer season, meanwhile oestradiol-17 concentration was significantly ($P < 0.05$) higher during winter season than other seasons of the year. Ovary weight, number of the corpora lutea (CL) and number of the normal follicles in the left were significantly ($P < 0.05$) higher than the right ovary, while the number of the atretic follicles in the right was significantly ($P < 0.05$) higher than the left ovary. Oocyte recovery and oocyte status (COC's, PDCO, ECO and DCO) in the left ovary were significantly ($P < 0.05$) higher than the right one. In respect to ovary side, AST, ALT, ALP, ACP, sodium and testosterone concentration of follicular fluid in the left ovary were significantly ($P < 0.05$) lower than the right one. Cholesterol, potassium, calcium, inorganic phosphorus and oestradiol-17 concentrations in the left were significantly ($P < 0.05$) higher than the right ovary. In the second experiment, results revealed significantly ($P < 0.05$) higher cumulus expansion, meiosis metaphase I (MI) and metaphase II (MII) than the non-breeding season. When the type of culture media there was no differences in cumulus expansion except with basal medium which produce the lowest incidence in both breeding and non-breeding season. In breeding season, TCM-199 medium showed the highest rate ($P < 0.05$) of MII oocytes, while in non-breeding season, TCM-199 and Ham's F-10 media showed the highest rates ($P < 0.05$) of MII oocytes.

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1. Introduction

Camels are induced ovulations and exhibit follicular cycles with follicles developing and regressing successively and ovulation will occur only when mating takes place (Elias *et al.*, 1984 and Ismail, 1987). Both the dromedary and bactrian camels are regarded as seasonal breeders, with a relatively short breeding season, based on the seasonal, distribution of

births and the status of ovarian activity (Shalash, 1980). Outside the breeding season, mating activity ceases and the ovaries are inactive or only have a few small follicles. However, there are conflicting reports about the beginning and length of the seasonal activity in the dromedary, increased breeding activity has been reported to occur in March and August in Sudan (Musa and Abusineina, 1978), December to March in Pakistan

(Yasin and Wahid, 1957), December to April in Egypt (Shalash, 1987) and from November to April in most of Arabian countries (Tibary and Anouassi, 1996). This is generally during the period of low climatic temperature, rain and better grazing conditions.

Application of assisted reproductive technologies such as artificial insemination, embryo transfer and *in vitro* production of embryos, which as in most domestic species could offer an opportunity to better understanding of factor that regulate reproduction in camels. In this respect, the application of the *in vitro* embryo production technology can facilitate the study of basic mechanisms regulating reproduction in camels.

Ovaries from slaughter house being the cheapest and most abundant source of oocytes are used for large scale production of mature oocytes in most of the animal species. As such, extensive studies on *in vitro* oocyte maturation of many domestic species have lead to improved culture conditions, so that a large percentage of oocytes successfully complete nuclear maturation (Eppig, 1991).

The *in vitro* maturation technique (*IVM*) needs a large number of good quality oocytes, which mainly depend upon the available number of follicles on the ovary in addition to the method of recovery. The regulation of oocyte maturation not only affected the proportion of oocytes capable of undergoing maturation, but also their subsequent fertilization and development (Bavister *et al.*, 1992). Few authors have studied *in vitro* maturation and fertilization of camel oocytes.

Therefore, the present study included two experiments. The first experiment, aimed to investigate the effect of different seasons of the year on ovarian activity of the dromedary she-camel. The second experiment, intended to define the effects of various maturation media during breeding and non-breeding seasons on the *in vitro* maturation of she-camel oocytes.

2. Materials and Methods

The present study was conducted in the Laboratory of Physiology, Department of Animal Production, Faculty of Agriculture, Mansoura University, in co-operation with Animal Production Research Institute, Dokki, Giza, Egypt.

The experimental work was carried out in the Private Camel's Farm, Belbies City, Sharkiya Governorate, located in the North Eastern part of the Nile Delta (30 °N). A total number of 220 clinically healthy she-camel was used in this study. The age of these camels varied from 5 to 10 years and their weights were approximately 500-600 kg. The present work included two experiments. The first experiment,

aimed to investigate the effect of different seasons of the year on follicular fluid components (AST, ALT, ALP, ACP, cholesterol, sodium, potassium, calcium, inorganic phosphorus, testosterone and oestradiol-17 β hormone) and ovarian activity (ovary weight, number of corpora lutea, number of oocytes and oocyte status) either in the right or left ovary of the dromedary camel. The second experiment designed to define the effects of various *in vitro* maturation media (TCM 199, Ham's F-10, Basal medium and Hank's) on the maturation rate of she-camel oocytes during the breeding and non-breeding seasons.

Minimum and maximum values of air temperature (°C), relative humidity (%), temperature-humidity index (THI) and length of daylight (hours) of the different seasons of the year are shown in Table 1. The temperature - humidity index (THI) was estimated according to Livestock and Poultry Heat Stress Indices (LPHSI, 1990), using the following formulae:

$$\text{THI} = \text{db}^\circ\text{F} - (0.55 - 0.55 \text{ RH}) (\text{db}^\circ\text{F} - 58.00)$$

where db °F=dry bulb temperature in Fahrenheit and RH=relative humidity (RH% / 100). The obtained values of THI were classified as follows: less than 72=absence of heat stress, 72 to < 74 moderate heat stress, 74 to < 78=severe heat stress and over 78 very severe heat stress.

First experiment

1. Ovarian activity:

1.1. Ovaries collection:

A total number of 440 ovaries collected from 220 clinically healthy she-camels was used in this study. Two ovaries (right and left) from each camel were collected immediately after slaughtering within 30-60 minutes and washed by sterile warm normal saline (0.9% NaCl) containing 100 IU penicillin G-sodium and 100 μg streptomycin sulfate /ml. The ovaries were kept in pairs in plastic bags containing saline, then transported to the laboratory in a thermos containing sterile normal saline at 30 - 35°C. Weight and number of corpora lutea, follicles, oocytes and oocytes status per ovary, were recorded. The ovaries were excised and submerged in *in vitro* fertilization (*IVF*) dishes with saline solution (0.9% NaCl). Number of oocytes was recorded from the normal visible follicles (5-10 mm) on ovaries existed from camel then punctured and the content was expelled into a disposable Petri dish (30X60mm). The follicular fluid was centrifuged at 600 g for 15 minutes to remove the cellular debris and the supernatant fluid was pooled in each class separately stored at - 20 °C to fulfill all required biochemical analysis.

Table (1): Mean air temperature (°C), relative humidity (%), temperature-humidity index (THI) values and daylight length, during the different seasons of the year.

Seasons	Air temperature (°C)		Relative humidity (%)		Temperature-humidity index (THI)		Length of daylight (hours)
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	
Winter	8.86±0.21	19.15±0.35	48.62±0.35	64.33±1.15	45.11	64.81	11.55
Spring	13.60±0.18	24.16±0.18	37.41±0.43	52.64±1.21	55.96	70.93	14.13
Summer	20.84±0.32	34.30±0.46	38.83±0.48	53.66±0.95	65.64	84.63	15.24
Autumn	15.43±0.12	28.62±0.42	42.67±0.62	58.42±1.32	59.21	77.68	13.00

1.2. Ovarian weight (g):

After removal of the extraneous tissues, each ovary (right or left) was weighed using an electric balance.

1.3. Number of follicles and corpora lutea:

Immediately after slaughter, ovaries were removed and all the normal visible follicles (5-10 mm) or corpora lutea either left or right ovaries were counted.

1.4. Follicle type:

The follicle was differentiated according to the nature of the contained follicular fluid as follow:

- Normal follicles: were target, transport, almost spherical, easily squeezable and thick wall.
- Atretic follicles: were opaque, nearly spherical and relatively thin walled.

2. Follicular fluid components:

AST, ALT enzyme activities were determined colourimetrically using the method described by Reitman and Frankle (1957), while ALP and ACP activities and cholesterol concentration were determined colourimetrically using commercial kits purchased from Bio-Merieux (Marcy L'Eltoile, Charbonnières, Les Bains, France) according to Graham and Pace (1967). Inorganic phosphorus, sodium, potassium and calcium concentrations were determined according to the method described by Kuttner and Liechtenstein (1930), Trinder (1951), Sunderman Jr. Sunderman (1958) and Gindler (1972), respectively. Testosterone and oestradiol-17 β hormones were determined by Radiimmunoassay Technique (RIA) using commercial kits (Diagnostic Products Corporation, Los Angeles, USA).

3. Oocytes collection:

Oocytes were collected using aspiration from the antral follicles (5–10 mm in diameter) either left or right ovary individually using 5 ml syringe and 20 gauge needle. Before commencing aspiration, the needle and syringe are first primed with approximately

0.25 ml of aspiration medium. After aspiration, the contents of the syringe were slowly dispelled into sterile Petri dishes (30x60mm) with minimum disruption of the cumulus oocytes complex. Repeated aspirations of follicles were performed to collect oocytes into the syringe. Number of oocytes recovered from follicles into each of right or left ovaries was recorded using Stereo-microscope.

3. 1. Oocytes recovery:

Oocytes yield from aspiration of the follicles in each of right or left ovaries was recorded. The recovery rate was determined as the percentage of oocytes in proportion to each of the total vesicular follicles according to Mayer *et al.* (1986) as the following formulae:

$$\text{Recovery rate} = \frac{\text{No. of oocytes recovered}}{\text{No. of vesicular follicles}} \times 100$$

3.2. Oocytes evaluation:

The oocytes were evaluated in respect to both investment and ooplasm granulation as the method described by Madison *et al.* (1992).

3.2.1. Cumulus evaluation

a. Compact cumulus oocytes complexes (COC's) :

Oocytes with complete compact dense cumulus oophorus more than 3 layers (grade I).

b. Partially denuded cumulus oocytes (PDCO) :

Oocytes with compact cumulus layer not completely surrounding the oocyte or less than 3 layers (grade II).

c. Expanded cumulus oocytes (ECO) :

Oocytes surrounded by expanded layers of cumulus cells appearing as scattered clumps in the matrix (grade III).

d. Denuded cumulus oocytes (DCO):

Oocytes enclosed only by the zona pellucida without cellular investment (grade IV).

3.2.2. Ooplasm evaluation:

a. Even ooplasm:

Granulation of ooplasm given the oocytes a dusty appearance and the ooplasm evenly fill the zona pellucida.

b. Uneven ooplasm:

Granules clumped or uneven distributed in ooplasm and the ooplasm remarkably fills the zona pellucida.

c. Shrunken ooplasm:

Ooplasm shrunken away from the zona pellucida or not evenly filling the zona. Ooplasm also looks degenerated with fragment empty zona pellucida.

The oocytes of category I and II and evenly granulated dark ooplasm were selected to undergo *in vitro* maturation (usable oocytes), but category III and IV were discarded (unusable oocyte).

Second experiment

In vitro maturation of camel oocytes:

1. Media:

Four types of maturation media (TCM-199, Ham's F-10, Hank's and Basal media) were used for oocytes washing and maturation obtained in a liquid form (from Egyptian Organization for Biological Product and Vaccine, Agoza) and stored in the refrigerator at 5°C till usage.

Preparation of media:

All media (TCM-199, Ham's F-10, Hank's and Basal media) supplemented with 10 mg L-glutamine, 100 IU Penicillin G-Sodium and 50 µg Streptomycin/100ml /ml were used. Value of pH was measured by pH meter and adjusted to pH 7.4 using NaOH (Sigma, Chemical P.O Box 14508 ST. Louis, MO 63178, USA). Each medium was sterilized using 0.2 µm millipore filter and equilibrated in CO₂ incubator (5% CO₂) with relative humidity of 38.5 - 39°C for at least 2 hours prior to use.

2. Cultivation of oocytes:

The selected oocytes were collected from the left ovary (according to the results of the first experiment) and washed three times in all the maturation media using line polished Pasteur pipette before being injected finally in four wells culture dishes, each containing 500-750 µl of the culture media.

3. Assessment of maturation:

The judgment of oocytes maturation was based on cumulus expansion and nuclear maturation.

3.1. Cumulus expansion:

The cumulus expansion was determined after oocytes incubation under Stereo microscope. The

criteria of assessing the cumulus expansion was done according to Chauhan *et al.* (1999) as follow:

- a. Expanded cumulus : cell mass was expanded away from the zona pellucida.
- b. Non expanded cumulus : cell mass was tightly adherent to the zona pellucida.

3.2. The nuclear maturation:

For the judging of the nuclear maturation, cumulus compact oocytes complexes (COC's) were transferred to small plastic tube containing 3% sodium citrate solution followed by repeated agitation for the denudation of the oocytes (Carolan *et al.*, 1994). The contents of the tube were transferred to a new 35 mm Petri dish and the demanded oocytes were mounted on a glass slide with a cover slip supported by droplets of paraffin Vaseline mixture. Thereafter, oocytes were fixed and cleared with ethanol acetic acid 3:1 at 4 °C for 24 h , and stained with aceto-orcein (1% orcein in 40% acetic acid) for 25 minutes. After that oocytes were rewashed with a fresh fixative and examined under light microscope (Ganguli *et al.*, 1998). The stage of nuclear maturation was described as follows:

- a. Immature: Germinal vesicle stage with intact nucleus.
- b. Intermediate: Paired or bivalent chromosomes were observed within nucleus of the oocyte (Metaphase I).
- c. Mature: Two groups of unequally spread chromosomes were observed and the polar body set was clustered together (Metaphase II). Thereafter, the oocytes were categorized as post maturation cumulus expansion (Plate 1), germinal vesicle (GV, Plate 2), metaphase I (M1, Plate 3) and metaphase II (MII, Plate 4).

Data were statistically analyzed using least squares Analysis of Variance according to Snedecor and Cochran (1982). Percentage values were transformed to arc-sin values before being statistically analyzed. Duncan's multiple range test (Duncan, 1955) were used for the multiple comparisons.

3. Results and Discussion:

First experiment

1. Temperature-humidity index (THI):

The temperature-humidity index (THI) estimated in Table 1 indicated exposure of she-camel to very severe heat-stress during summer (non-breeding season), severe heat stress during autumn and absence of the heat-stress during winter and spring seasons (breeding season).

2. Ovarian activity:

2.1. Ovary weight and CL number:

Data presented in Table 2 showed that the effect of season of the year on ovary weight was significant ($P < 0.05$). The highest ($P < 0.05$) value of ovary weight was recorded during autumn and winter and the lowest ($P < 0.05$) values was recorded during summer and spring seasons. Similar trends were recorded by Abdoon and Omima (2006) and Sarhan (2007).

With regard to ovary side, the left and right ovary weight in the dromedary she-camel was significant ($P < 0.05$). In the left ovary, the highest ($P < 0.05$) weight was obtained during autumn followed by those of winter and the lowest ($P < 0.05$) weight during summer. While, in the right ovary, the highest and lowest weights were recorded during winter and summer, respectively. These results are in agreement with those of Yahaya *et al.* (1999) who showed that the right ovary was heavier ($P < 0.01$) and had more follicular fluid ($P < 0.01$) than left ovaries. In general, ovary weight, increases with ovarian activity (Djang *et al.*, 1988 and El-Wishy, 1992). Ovary side showed significantly effects on the ovary weight although ovary weight was significantly ($P < 0.05$) higher in the left than in the right ovary during all seasons of the year.

The mean number of CL per ovary of dromedary she-camel was significantly ($P < 0.05$) between seasons. The highest ($P < 0.05$) number of CL was obtained during autumn followed by that of spring and the lowest ($P < 0.05$) during summer and winter seasons. The present results are in agreement with those of Yahaya *et al.* (1999) and Sarhan (2007) who showed that, the number of corpora lutea increased during November and December (breeding season). Ovaries examined had 2 active corpora lutea and no ovaries were found with 3 corpora lutea. Abdoon (2001) showed that ovaries without a CL possessed significantly ($P < 0.01$) more ovarian follicles and more ($P < 0.05$) small and large follicles.

Ovary side had significantly ($P < 0.05$) effects on the ovary number of CL, being higher in the left than in the right ovary during all seasons of the year.

In general, it is interested to notice that, superiority of the oocytes recovery in both left and right ovaries of the dromedary she-camels was recorded in the breeding season (winter, autumn and spring) as compared to the non-breeding (summer) season. These probably may be due to that the gonadotropic hormonal balance was in favor of the follicular growth stimulation oocyte status in the breeding season but is not in favor of ovulation process.

2.2. Follicles number:

Data presented in Table 2 showed the effect of the number of the normal follicles was significantly

($P < 0.05$) increased during spring as compared to other seasons. While, the number of the atretic follicles was significantly ($P < 0.05$) increased during summer as compared to other seasons. In respect to ovary side, the total number of the normal follicles in the left ovary was significantly ($P < 0.05$) higher and significantly ($P < 0.05$) lower the atretic follicles than the right one. Meanwhile, the total number of follicles was significantly ($P < 0.05$) higher during summer and winter than spring and autumn seasons. Similar trends were reported by Amer (2004) and Sarhan (2007).

The number of follicles of the dromedary she-camel on the left and right ovaries was significant ($P < 0.05$). The highest number ($P < 0.05$) of the normal follicles was obtained during spring and the lowest ($P < 0.05$) during summer in the right ovary. While, in the left ovary, the highest ($P < 0.05$) number of the normal follicles was recorded during winter and the lowest ($P < 0.05$) during summer. Concerning the atretic follicles, the highest ($P < 0.05$) number of the atretic follicles was obtained during summer and the lowest ($P < 0.05$) during spring in the right or left ovary. These results are in agreement with those of Abdoon (2001), Abdoon and Omamia (2006) and Sarhan (2007) who showed that, the number of small, medium, large and the total number of ovarian follicles were higher ($P < 0.01$) during the breeding than non-breeding season. Similar trends were reported by Amer (2004) and Zeidan *et al.* (2008).

2.3. Oocytes recovery:

Table 3 showed that the mean number of oocytes per ovary collected was significantly ($P < 0.01$) lower during summer than the other seasons. Abdoon (2001) showed that, the total recovery of oocytes with compact cumulus was greater ($P < 0.01$) during the breeding season. Similar trends were reported by Amer (2004) and Zeidan *et al.* (2008).

In respect to ovary side, the recovery rate of oocytes was significantly ($P < 0.05$) higher in the left than the right ovary during all the different seasons of the year. Similar trends were reported by Amer (2004) and Zeidan *et al.* (2008).

2.4. Oocytes status:

Data presented in Table 3 showed that the means cumulus oocytes complexes (COC's) and partially denuded cumulus oocytes (PDCO) of the dromedary she-camels increased significantly ($P < 0.05$) during spring, autumn and winter as compared with the summer season. The maximal ($P < 0.05$) numbers of COC's and PDCO was recorded in autumn season and the minimal ($P < 0.01$) numbers was recorded in summer one. The expanded cumulus oocytes (ECO) and denuded cumulus oocytes (DCO) were significantly ($P < 0.05$) higher during spring and

winter than the summer and autumn seasons. The maximal ($P < 0.05$) numbers of the ECO was recorded during spring and the minimal ($P < 0.05$) numbers was recorded in autumn season. Meanwhile, the maximal ($P < 0.05$) numbers of the DCO was recorded in winter and the minimal ($P < 0.05$) numbers was recorded in summer. Similarly, Amer (2004) and Sarhan (2007) found that the highest numbers of COC's and PDCO was recorded during autumn and winter and the lowest numbers was recorded during summer and spring seasons. While, the highest ECO and DCO were

recorded during spring and winter and the lowest during summer and autumn seasons.

Regarding side of ovary, the numbers of COC's , ECO, PDCO and DCO oocytes of the dromedary she-camels were significantly ($P < 0.05$) higher in the left than the right ovary during the different seasons of the year. Sarhan (2007) showed that the ovary side had significant ($P < 0.01$) effect on the oocytes status being higher in the breeding than the non-breeding season. Similar trends were reported by Amer (2004) and Zeidan *et al.* (2008).

Table (2): Means of the right and left ovaries weight, number of corpora lutea and number of the normal and atretic follicles of the dromedary she-camel as affected by different seasons of the year.

Item	Seasons of the year											
	Spring		Mean	Summer		Mean	Autumn		Mean	Winter		Mean
	LO	RO		LO	RO		LO	RO		LO	RO	
Ovary weight (g)	5.07 ^a	4.48 ^b	4.78 ^B	5.02 ^a	4.46 ^b	4.74 ^B	5.51 ^a	4.84 ^b	5.18 ^A	5.49 ^a	5.06 ^b	5.28 ^A
	±	±		±	±		±	±		±	±	
	0.84	0.76		0.74	0.71		0.72	0.58		0.71	0.65	
No. of corpora lutea/ovary	2.92 ^a	2.54 ^b	2.73 ^A	2.25 ^a	1.63 ^b	1.94 ^B	3.00 ^a	2.57 ^b	2.79 ^A	2.23 ^a	1.85 ^b	2.04 ^B
	±	±		±	±		±	±		±	±	
	0.25	0.15		0.65	0.38		0.74	0.37		0.34	0.42	
Total	5.28 ^A			3.88 ^C			5.57 ^A			4.08 ^B		
No. of the normal ovarian follicles	14.09 ^a	10.64 ^b	12.37 ^A	6.37 ^a	4.25 ^b	5.31 ^D	12.43 ^a	7.28 ^b	9.86 ^C	16.08 ^a	7.07 ^b	11.58 ^B
	±	±		±	±		±	±		±	±	
	1.88	1.62		0.26	0.93		1.13	1.51		1.05	1.57	
Total	24.73 ^A			10.62 ^C			19.71 ^B			23.15 ^A		
No. of the atretic ovarian follicles	1.72 ^b	2.86 ^a	2.29 ^D	13.36 ^b	16.82 ^a	15.09 ^A	4.87 ^b	5.37 ^a	5.12 ^C	8.07 ^b	8.85 ^a	8.46 ^B
	±	±		±	±		±	±		±	±	
	0.28	0.34		1.47	0.84		0.16	0.83		0.51	0.74	
Total	4.58 ^D			30.18 ^A			10.24 ^C			16.92 ^B		
Total number of follicles	29.31 ^C			40.80 ^A			29.95 ^C			40.07 ^A		

Means bearing different letters within the same row, differ significantly ($P < 0.05$). RO : Right ovary.
LO: Left ovary

Table (3) : Means of recovery rate and oocytes status in the right and left ovaries of the dromedary she-camel as affected by different seasons of the year.

Item	Seasons of the year											
	Spring		Mean	Summer		Mean	Autumn		Mean	Winter		Mean
	LO	RO		LO	RO		LO	RO		LO	RO	
No. of ovaries	11	11	11	13	13	13	7	7	7	8	8	8
Oocytes recovery	4.00 ^a	1.44 ^b	2.72 ^B	1.62 ^a	0.33 ^b	0.98 ^D	9.99 ^a	5.14 ^b	7.57 ^A	4.13 ^a	3.50 ^b	3.82 ^C
Investments :												
Compact cumulus oocytes complexes	1.00 ^a	0.18 ^b	0.59 ^C	0.23 ^a	0.10 ^b	0.17 ^D	8.71 ^a	3.72 ^b	6.22 ^A	1.63 ^a	1.26 ^b	1.45 ^B
	±	±		±	±		±	±		±	±	
	0.27	0.13		0.09	0.00		0.34	0.85		0.41	0.47	
Partially denuded cumulus oocytes	0.90 ^a	0.09 ^b	0.50 ^C	0.23 ^a	0.00 ^b	0.12 ^D	1.28 ^a	1.00 ^b	1.14 ^A	0.87 ^a	0.55 ^b	0.71 ^B
	±	±		±	±		±	±		±	±	
	0.09	0.09		0.12	0.00		0.03	0.05		0.03	0.02	
Expanded cumulus oocytes	1.55 ^a	0.81 ^b	1.18 ^A	0.46 ^a	0.23 ^b	0.35 ^C	0.28 ^a	0.00 ^b	0.14 ^D	0.87 ^a	0.50 ^b	0.69 ^B
	±	±		±	±		±	±		±	±	
	0.47	0.23		0.18	0.12		0.18	0.00		0.41	0.27	
Denuded cumulus oocytes	0.55 ^a	0.36 ^b	0.46 ^B	0.70 ^a	0.00 ^b	0.35 ^C	0.14 ^a	0.00 ^b	0.07 ^D	1.13 ^a	0.62 ^b	0.88 ^A
	±	±		±	±		±	±		±	±	
	0.21	0.21		0.07	0.00		0.14	0.00		0.45	0.18	

Means bearing different letters within the same row, differ significantly ($P < 0.05$). RO : Right ovary.
LO : Left ovary

3. Follicular fluid components:

3.1. Enzymatic activities:

The effects of season of the year and ovary side on the follicular fluid (FF) components of the dromedary camels are presented in Table 4.

The effect of season of the year on AST, ALT, ALP and ACP enzymes activity in FF of the camels was significantly ($P < 0.05$) higher during summer than autumn, winter and spring seasons. The highest ($P < 0.05$) value of AST, ALT and ALP enzymes was recorded in summer and the lowest ($P < 0.05$) values were recorded in spring season. While, the ACP enzyme showed higher activity during summer than autumn, spring and winter, since lowest value in autumn season. These results are in agreement with those of Mabrouk *et al.* (1991) and Amer (2004).

Regarding the ovary side, the right ovary showed significantly ($P < 0.05$) higher activity of AST, ALT, ALP and ACP enzymes than the left one. The active granulosa cells shared to some extent in adding both AST and ALT enzymes to the follicular fluid of she-camel (Mabrouk *et al.*, 1991). The activity of AST and ALT enzymes in the right ovary might be due to more atrophied and degenerated granulosa cells and the enlargement of cystic size in atretic follicles and to the presence of more active membrane granulosa cells in normal follicles (Abdel Ghaffar *et al.*, 1995). Amer (2004) and Zeidan *et al.* (2008) confirmed that the AST, ALT, ACP and ALP activities in the right ovary were significantly higher than the left one. In contrast, ALT, AST, ALP and ACP enzyme activities in the normal follicular fluid of women was higher than in cystic follicles (Causing *et al.*, 1972).

In general, our results showed that the follicular fluid contains high levels of transaminases and phosphatases enzymes which increase with follicular development. So, it is suggested that transaminases and phosphatases enzymes may affect ovarian steroidogenesis. The role of granulosa cells in the contribution of these enzymes may be accepted. Likewise, the significant increase in ALP enzyme activity in the follicular fluid might be an indication of atrophy due to lysosomal enzymes that affected the phosphorylated receptors which would lead to atresia (Wise, 1987 and Abdel-Ghaffar *et al.*, 1995).

3.2. Cholesterol concentration:

The effect of season of the year had significantly ($P < 0.05$) effect on cholesterol concentration in FF of the dromedary she-camels. The highest ($P < 0.05$) value of follicular fluid cholesterol was recorded during autumn and the lowest ($P < 0.05$) value was recorded during summer season. Similar trend was reported by Amer (2004), Sarhan (2007) and Zeidan *et al.* (2008) in the dromedary camels. Total

cholesterol concentration depends on the environmental and seasonal variations (Sinha *et al.*, 1981). The seasonal variations in cholesterol concentration may be due to the type of feed offered during different seasons of the year. During breeding season (winter), the green fodder was barseem since barseem is a rich source of steroids (Salem, 1980). In addition, decrease of cholesterol during non-breeding season (summer) may be due to lower thyroid activity during rise of environmental temperature which influences cholesterol level.

With regard to ovary side, cholesterol concentration in the right ovary was significantly ($P < 0.05$) lower than the left one during the different seasons of the year. Similar trends were reported by Amer (2004) and Zeidan *et al.* (2008).

3.3. Minerals concentration:

The effects of different seasons of the year on calcium in FF of the dromedary she-camels was significantly ($P < 0.05$) higher during autumn and winter than spring and summer seasons. The highest ($P < 0.05$) value of follicular fluid calcium concentrations was recorded during autumn and the lowest ($P < 0.05$) value during summer. The increase of calcium concentration at winter may be due to the high calcium values of barseem during breeding season (Ayoub *et al.*, 1972). Similar trends were reported by Amer (2004) and Zeidan *et al.* (2008). These results might be attributed to the atrophy and degenerative changes in the granulosa cells during the non-breeding season than the breeding one. This clearly shows to what extent the higher stressful temperature in summer during the non-breeding season exerted unfavorable effects on ovarian activity in terms of lower stimulatory follicular growth than the breeding one.

The effect of season of the year was significantly ($P < 0.05$) higher on sodium concentration in FF during summer than autumn, winter and spring seasons. The highest ($P < 0.01$) value of sodium in FF was recorded during summer and the lowest ($P < 0.01$) value was found during spring season. Similar trend was reported by Amin (1993) and Sarhan (2007). Amer (2004) found also that, sodium concentration of the dromedary she-camels was significantly higher during summer than spring, autumn and winter seasons. The increase of sodium concentration during the heat of summer which indicates a very effective mobilization of the intracellular fluids into extracellular spaces (Rathore, 1986). In addition, these results may be attributed to the combined effect of sodium and chloride absorption from the alimentary tract and kidney, respectively, under the effect of aldosterone hormone which had higher level in summer and this

was accompanied by an increase of plasma sodium level (Yagil and Etzion, 1979).

The effects of different seasons of the year on FF potassium and inorganic phosphorus concentrations were significantly ($P < 0.05$) lower during summer than autumn, winter and spring seasons. The highest ($P < 0.05$) value of FF potassium and inorganic phosphorus concentrations was recorded during spring and winter and the lowest ($P < 0.05$) value was recorded during summer season. Similar trend was reported by Amer (2004) and Zeidan *et al.* (2008) in the dromedary camels.

With regard to ovary side, follicular fluid sodium concentration was significantly ($P < 0.01$) higher, while calcium, potassium and inorganic phosphorus concentrations were significantly ($P < 0.05$) lower in the right than the left ovary with the different seasons of the year. Similar results were recorded by Amer (2004) and Sarhan (2007) in the dromedary camels. These results may indicate early follicular degeneration of the atretic follicles at the right ovary than in the normal follicles of the left ovary.

Table (4) : Means of the follicular fluid components in the left and right ovaries of the dromedary she-camels during different seasons of the year.

Item	Seasons of the year										Mean	
	Spring		Mean	Summer		Mean	Autumn		Mean	Winter		
	LO	RO		LO	RO		LO	RO		LO		RO
Aspartate-aminotransferase (U/l)	39.41 ^b	68.01 ^a	53.71 ^C	112.38 ^b	129.05 ^a	120.72 ^A	76.18 ^b	96.71 ^a	86.45 ^B	74.74 ^b	95.37 ^a	85.06 ^B
	±	±		±	±		±	±		±	±	
	2.27	1.61		6.21	8.59		3.48	3.43		3.67	4.92	
Alanine-aminotransferase (U/l)	6.74 ^b	37.33 ^a	22.04 ^C	38.86 ^b	52.40 ^a	45.63 ^A	33.13 ^b	38.37 ^a	35.75 ^B	35.75 ^b	39.50 ^a	37.11 ^B
	±	±		±	±		±	±		±	±	
	2.15	2.74		1.68	1.00		1.19	0.23		1.51	1.67	
Alkaline phosphatase (U/l)	227.21 ^b	342.75 ^a	284.98 ^C	321.57 ^b	369.12 ^a	345.35 ^A	310.54 ^b	324.76 ^a	317.65 ^B	274.83 ^b	300.74 ^a	287.79 ^C
	±	±		±	±		±	±		±	±	
	20.82	22.04		19.01	15.23		08.12	2.26		0.52	64.14	
Acid phosphatase (U/l)	2.16 ^b	5.01 ^a	3.59 ^B	2.70 ^b	6.24 ^a	4.47 ^A	2.12 ^b	2.50 ^a	2.31 ^C	2.80 ^b	4.01 ^a	3.41 ^B
	±	±		±	±		±	±		±	±	
	0.19	0.46		0.58	0.66		0.03	0.03		0.15	0.79	
Cholesterol (mg/dl)	136.33 ^a	113.33 ^b	124.83 ^A	121.33 ^a	98.66 ^b	110.00 ^B	135.66 ^a	125.66 ^b	130.66 ^A	131.00 ^a	126.00 ^b	128.50 ^A
	±	±		±	±		±	±		±	±	
	12.46	2.61		09.39	10.93		10.25	5.18		2.56	4.44	
Calcium (mg/dl)	7.63 ^a	6.10 ^b	6.87 ^C	7.51 ^a	6.46 ^b	6.99 ^C	9.60 ^a	9.00 ^b	9.30 ^A	9.46 ^a	8.43 ^b	8.95 ^B
	±	±		±	±		±	±		±	±	
	1.61	1.98		0.16	0.91		0.55	0.17		0.93	1.94	
Sodium (mg/dl)	78.33 ^b	92.33 ^a	85.33 ^C	133.00 ^b	139.50 ^a	136.25 ^A	112.30 ^b	135.33 ^a	123.82 ^B	84.00 ^b	89.00 ^a	86.50 ^C
	±	±		±	±		±	±		±	±	
	13.39	5.37		4.05	1.16		2.03	2.91		8.55	3.22	
Potassium (mg/dl)	7.33 ^a	6.60 ^b	6.97 ^A	3.20 ^a	4.10 ^b	3.65 ^C	5.56 ^a	4.40 ^b	4.98 ^B	8.30 ^a	5.93 ^b	7.12 ^A
	±	±		±	±		±	±		±	±	
	0.53	1.12		0.81	0.17		0.34	0.31		0.09	0.78	
Inorganic phosphorus (mg/dl)	8.26 ^a	6.63 ^b	7.45 ^A	6.53 ^a	4.93 ^b	5.73 ^C	6.83 ^a	6.36 ^b	6.60 ^B	6.86 ^a	6.53 ^b	6.70 ^B
	±	±		±	±		±	±		±	±	
	0.21	0.27		0.62	0.08		0.07	0.07		0.08	0.24	
Testosterone (pg/ml)	11.49 ^b	16.51 ^a	14.00 ^D	19.89 ^b	82.99 ^a	51.44 ^A	15.32 ^b	20.78 ^a	18.05 ^C	12.58 ^b	27.59 ^a	20.09 ^B
	±	±		±	±		±	±		±	±	
	1.18	1.03		0.25	2.74		1.29	0.79		0.17	3.23	
Oestradiol 17- (pg/ml)	138.46 ^a	122.56 ^b	130.51 ^A	138.46 ^a	105.27 ^b	121.87 ^B	136.51 ^a	112.38 ^b	124.45 ^B	142.84 ^a	121.18 ^b	132.01 ^A
	±	±		±	±		±	±		±	±	
	22.18	41.25		41.25	19.34		28.12	25.16		36.15	28.11	

Means bearing different letters within the same row, differ significantly ($P < 0.05$).

RO : Right ovary.

LO : Left ovary

3.4. Hormonal profiles:

The effects of season of the year on testosterone concentration in FF of the dromedary she-camel were significant ($P < 0.05$), being higher during summer than spring, autumn and winter seasons. The highest ($P < 0.05$) concentration of testosterone was recorded during summer and the lowest ($P < 0.05$) concentration during spring. Similar trend was reported by Amer (2004) and Zeidan *et al.* (2008) in the dromedary camels. Heller and Ross (1979) showed that, there was a distinctive evidence of follicular steroid modulation of synthesis by androgens and a characteristic feature of the atretic follicles was high concentration of androgens in the follicular fluid. While, Abdel Ghaffar *et al.* (1995) indicated that a highly significant increase in testosterone content in FF of the atretic follicles than in normal follicular fluid in she-camel. These results might be due to the increased thickness of thecal layers which might be the source of androgens and the last layer affected the atresia process (Moor, 1977). Moreover, as atresia progressed granulosa cells were degenerated and the thecal cells were hypertrophied (Mori *et al.*, 1982). This may indicate that concentrations of the androgens in FF were positively correlated with the androgen content of thecal cells (Grant *et al.*, 1989).

In respect to ovary side, testosterone concentration in FF was highly significant ($P < 0.05$). Testosterone hormone concentration was significantly ($P < 0.05$) higher in the right than in the left ovary during all seasons of the year. The high androgen concentration in the follicles might not always signify atretic status, because healthy follicles might transit through an androgen dominant phase before acquiring the capacity to synthesize increased amount of estrogen (Mc Natty *et al.*, 1984).

The effects of season of the year on oestradiol 17- concentration in FF in the dromedary she-camel were significant ($P < 0.05$), being higher during winter and spring than autumn and summer seasons. The highest ($P < 0.01$) value of the FF estradiol-17 concentration was recorded during winter and the lowest ($P < 0.01$) value during summer. These results are in agreement with those of Agarwal *et al.* (1987) who found that the oestradiol 17- concentration elevated during breeding (winter) and decreased during non-breeding season (summer). Also, Abd El-Azim (1996) showed that the highest level of oestradiol-17 was recorded in winter (130 pg/ml) and spring (117.42 pg/ml) and the lowest level in autumn (83.60 pg/ml) and summer (54.58-pg/ml). These results may be attributed to the involvement of estrogens in modulation of sexual behavior (McEwen, 1976) and testosterone secretion (Eiler and Graves, 1977) in the male one-humped camel. It is hypothesized that decreasing light hours and probably low temperature

might be instrumental in triggering the hypothalamic hypophysial axis as was observed in other short day breeders like sheep (Turek and Campbell, 1979). In addition, Bedrak *et al.* (1983) observed that the relative activity of several enzymes associated with testosterone and its conversion to estrogen in the blood plasma of male one-humped dromedary camel was significantly lower during the non mating season than that of the mating one.

In respect to ovary side, oestradiol 17- concentration was significantly ($P < 0.05$) higher in the left than in the right ovary during all seasons of the year. These results are in agreement with those of Amer (2004) and Zeidan *et al.* (2008).

Second experiment:

In vitro maturation:

The effects of breeding and non-breeding seasons with the different maturation media on cumulus expansion and nuclear maturation of the dromedary camel oocytes are shown in Table 5 and Plates 1, 2, 3 and 4.

Our results revealed highly significant ($P < 0.05$) increase in the number of oocytes collected during breeding season, that showed postmaturation cumulus expansion (Plate 1), meiosis metaphase I (Plate 3), metaphase II (Plate 4) and Plates 3 and 4 after cultivation of the oocytes. While, Plate 2 shows oocytes at germinal vesicle (GV) stage. Regarding the total rate of expansion of cumulus complexes after 24 hours of maturation, it was significantly ($P < 0.05$) higher in breeding season (80.7%) compared to the non-breeding season (66.5%). The incidence of oocytes reached MI and MII stages of nuclear maturation was significantly ($P < 0.05$) higher in breeding season (20.7 and 60.0 %, respectively) in comparison to the non-breeding season (27.0 and 39.5 %, respectively). These results are in agreement with those of Amer *et al.* (2003) who confirmed that, the rate -of nuclear maturation increased from 12 to 24 hrs and remained constant up to 36 hrs and the rates of metaphase-II oocytes were higher during breeding season than non-breeding one in dromedary camel.

Cultivation of the oocytes in TCM-199 showed cumulus expansion, MI and MII being 85.2, 17.0 and 68.2% compared with 69.4, 16.7 and 52.8 % in oocytes cultured in Basal medium and 87.5, 33.9 and 53.6 %, respectively in Ham's F-10 while, that were 81.3, 18.8 and 62.5%, respectively for oocytes cultured in Hank's medium. On the other hand, in non-breeding season the values were 66.7, 22.2 and 44.4%, 60.0, 20.0 and 40.0%, 75.0, 23.3 and 51.7 % and 60.0, 40.0 and 20.0% for TCM-199, Basal, Ham's F-10 and Hank's media, respectively. The highest ($P < 0.05$) rate of the non-matured oocytes was recorded with the Basal medium during breeding season and Basal and

Hank's media during the non-breeding season. Meanwhile, the non-matured oocytes rate was significantly ($P < 0.05$) higher during the non-breeding season than breeding one. Similar trends were reported by Amer (2004) and El-Harairy *et al.* (2006) in the dromedary camel.

When the type of culture media related to the rate of oocyte maturation, there was no differences in cumulus expansion except with Basal medium which produce the lowest ($P < 0.05$) incidence in breeding season and Basal and Hank's media in the non-breeding season. With all types of media, the rate of cumulus expansion and M II oocytes was significantly ($P < 0.05$) higher in breeding than non-breeding season. In breeding season, TCM-199 and Hank's media showed the highest ($P < 0.05$) rate of M II oocytes, whereas, Ham's F-10 medium showed the highest ($P < 0.05$) rate of MI oocytes. In non-breeding season, TCM-199 and Ham's F-10 showed the highest ($P < 0.05$) rates of M II oocytes, while, Hank's medium showed the highest ($P < 0.05$) rate of MI oocytes. The oocytes were cultivated for 36 hours which was the common period for cultivation of camel oocytes. Abdoon, (2001) cultured

of camel oocytes for 36 hours produced higher ($P < 0.01$) percentages of cumulus expansion and oocytes at MII. Similar trends were reported by Amer *et al.* (2003), Amer (2004) and Khalil (2009) in the dromedary camels.

4. Conclusion:

In conclusion, camel (*Camulm dromedaries*) showed better, follicular fluid components and oocyte status during breeding season (short daylight) than the non-breeding season (long day light). Ovarian activity showed higher in the left than right ovary. Acceptable dromedary camel oocytes maturation rate at metaphase II stage was obtained when oocytes were aspirated from the left ovary and cultured in TCM-199 or Ham's F-10 maturation media. Thus, season and maturation media had important role in improve the *in vitro* fertilization (*IVF*) and embryo transfer programs to enhance the fertilizing ability of camel. Therefore, further detailed investigations are needed to open away to improve the embryo production and transfer programs in the dromedary camel during both breeding, as well as, non-breeding season.

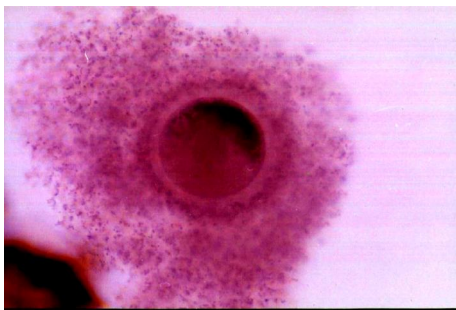


Plate. 1: Oocyte shows postmaturation cumulus expansion (x300).

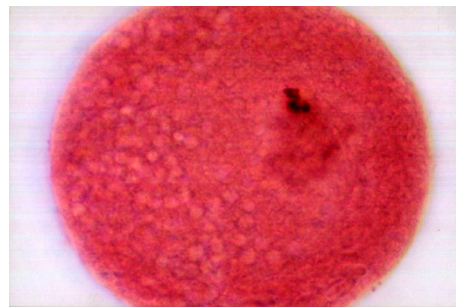


Plate. 2: Oocyte showing G V stage stained by Orcein stain (x1200).

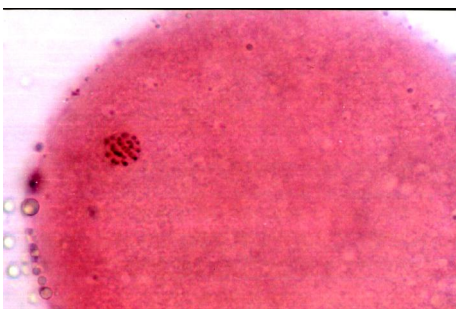


Plate. 3: Oocyte showing MI stained by Orcein stain (x1200).

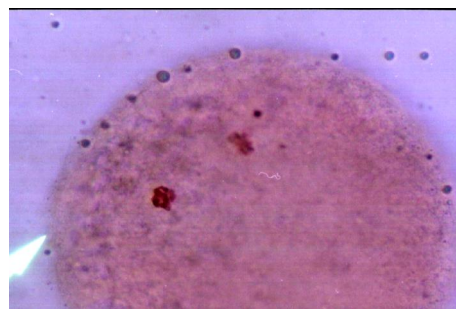


Plate 4: Oocytes showed MII stained by Orcein stain (x1200).

Table (5) : Percentage of cumulus expansion and nuclear maturation of the camel oocytes during breeding and non-breeding seasons with the different maturation media.

Maturation media	Breeding season					Non-breeding season				
	No. of oocytes	Matured oocytes			Non-matured oocytes	No. of oocytes	Matured oocytes			Non-matured oocytes
		Cumulus expansion	Metaphase I	Metaphase II			Cumulus expansion	Metaphase I	Metaphase II	
TCM-199 (%)	88	75 (85.2) ^a	15 (17.0) ^b	60 (68.2) ^a	13 (14.8) ^b	45	30 (66.7) ^b	10 (22.2) ^b	20 (44.4) ^{bc}	15 (33.3) ^b
Basal medium (%)	72	50 (69.4) ^b	12 (16.7) ^b	38 (52.8) ^b	22 (30.6) ^a	30	18 (60.0) ^b	6 (20.0) ^b	12 (40.0) ^c	12 (40.0) ^a
Ham's F-10 (%)	56	49 (87.5) ^a	19 (33.9) ^a	30 (53.6) ^b	7 (12.5) ^b	60	45 (75.0) ^a	14 (23.3) ^b	31 (51.7) ^a	15 (25.0) ^c
Hank's medium (%)	64	52 (81.3) ^a	12 (18.8) ^b	40 (62.5) ^a	12 (18.8) ^b	50	30 (60.0) ^b	20 (40.0) ^a	10 (20.0) ^d	20 (40.0) ^a
Means (%)	280	226 (80.7) ^A	58 (20.7) ^A	168 (60.0) ^A	54 (19.3) ^B	185	123 (66.5) ^B	50 (27.0) ^A	73 (39.5) ^B	62 (33.5) ^A

a, b, c, d : Means bearing different letters within the same column, differ significantly ($P < 0.05$).

A, B : Means bearing different letters within the same row, differ significantly ($P < 0.05$).

Corresponding Author

A.E.B. Zeidan

Animal Production Research Institute, Dokki, Giza, Egypt.

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