EFFECT OF BREEDING SEASON AND STEROIDOGENIC POTENTIAL OF GRANULOSA CELLS AND FOLLICULAR FLUID ON MATURATION OF CAMEL OOCYTES IN VITRO

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ABSTRACT

The effect of ovarian status and steroidogenic potential of granulosa cells and/or follicular fluid on the maturation of camel oocytes in vitro during rating and non-rating seasons were studied. The camel ovaries were collected immediately after slaughter. The oocytes were aspirated from follicles with 8-20 mm diameter for culture in maturation medium (MM) under different co-culture conditions. MM+No GCs (No Granulosa Cells), MM+FGCs (Fresh Granulosa Cells), MM+PGCs (Pre-incubated Granulosa Cells), MM+10%FF (Follicular Fluid) and MM+FGCs+10%FF. The obtained results revealed that, the distribution of small follicles on ovaries was significantly (P<0.01) higher than the medium and pre-ovulatory follicles. The different follicular populations with or without corpora lutea were significantly (P<0.01) higher during rating than non-rating season. During each season, the numbers of follicles were higher on ovaries with than without corpora lutea. There were no significant differences in the numbers of medium and large atretic follicles when two seasons were compared, whereas the medium atretic follicles showed higher significant rate (P<0.01) than large follicles during both seasons. The mean value of grad 1+2 oocytes was significantly (P<0.01) higher than grad 3+4 oocytes only during rating season, although the two groups were higher during rating season than non-rating one.

The highest rates of metaphase-II oocytes were observed after co-culture with FGCs during rating and non rating seasons, whereas the lowest rates were observed after co-culture with PGCs or FF. In all groups, the rates of metaphase-II oocytes were higher during rating season than non-rating one. The rate of nuclear maturation increased progressively with the duration of co-culture reaching the highest at 36 hrs during rating season. During non-rating season, the rate of nuclear maturation increased from 12 to 24 hrs and remained constant up to 36 hrs.

The potential of FGCs or PGCs to secrete testosterone and estradiol-17β in culture media was significantly (P<0.01) increased with the duration of co-culture reaching the highest with FGCs+FF at 36 hrs, with exception the level of estradiol-17β decreased after co-culture with PGCs. The potential of FGCs or PGCs to secrete progesterone increased progressively with the duration of co-cultring reaching the highest with PGCs at 36 hrs. The level of three hormones maintained significantly (P<0.01) higher during rating than non-rating season. In conclusion, conditioning of the culture media with GCs and FF had the potential to mature camel oocytes during non-rating as well as rating seasons. This will increase and improve the in-vitro fertilization and embryo-transfer programme in one-humped camel.

INTRODUCTION

The camel is an economic feeder as they can produce food and milk when other ruminants are not physiologically able. The camelidea are considered the perfect farm animal for arid areas because of their particular adjustment to drought conditions **(Shalash, 1965; Ismail et al., 1993)**. However, opportunities to improve reproductive efficiency in the camel are limited due to the continued use of traditional systems of reproductive management in most breeding herds. These old methods make it difficult to be sure that the optimum numbers of females are pregnant at the end of the season **(Cooper et al., 1990)**. Recently, the technique of in vitro maturation and subsequent fertilization and embryo-transfer can be employed to overcome some of these problems **(Ismail et al., 1993)**, especially to impregnate as many of females as possible at the start of the breeding season become pregnant.

Camels are induced ovulators and exhibit follicular cycles with follicles developing and regressing successively and ovulation will occur only when mating takes place **(Elias et al., 1984; Ismail, 1987)**. The main goals of this study included the effect of ovarian status and potential of granulosa cells and/or follicular fluid on the maturation of camel oocytes in vitro durin rating and non rating seasons.

MATERIALS AND METHODS

Ovaries collection:

The ovaries of camels were collected in pairs from Belbies and Cairo abattoir within 30 minutes after slaughter during rating and non-rating seasons, they placed in D-Phosphate Buffered Saline at 30-35°C. The ovaries were transported to the laboratory within 2 hrs. No information

on previous breeding history of these animals was known.

Follicular qualification:

During rating and non-rating seasons, all the ovarian follicules were qualified morphologically including:

- The classification of follicles into three follicular populations, small (3-6 mm), medium (7-15mm) and pre-ovulatory (>15 mm) follieles, and counting the number of follicles in each group.
- The follicular distribution on the ovaries with or without corpora lutea.
- The rate of follicular atresia including the medium and large follicles.

Oocytes recovery and assessment:

Only healthy follicles with 8-20 mm in diameter (according to the parameters described by **Pavlok et al. (1992)** were aspirated using 18-gauge needles. The aspirates were pooled into 60 mm petri dishes. All the aspirates were examined under Steriomicroscope for qualification and description of oocytes **(De Loos et al., 1992)**. The oocytes were classified into two groups (grad 1+2 and grad 3+4), and oocytes grad 1+2 were used for further investigations.

Collection of granulosa cells (GCs) and follicular fluid (FF) :

The collection of GCs was carried out directly after harvesting of oocytes from follicular aspirates. After centrifugation of the follicular aspirates at 2000 rpm/10 minutes, the FF was placed in small tubes at -20^0 untill use. The resulting pellet of GCs was washed twice with prewarmed culture medium and re-suspended in µl 400 Tissue Culture Medium-199 (TCM-199) and passed several times through 18-gauge needle to re-desperse GCs **(Gordon, 1995)**. The cellular concentration was adjusted to 4- 5x10^6 cells/ml before co-culture.

In vitro maturation:

The selected oocytes were washed twice in prewarmed TCM-199 (Sigma M-7523) with Earl's salts, 100 ug/ml L-glutamine and 50 ug/ml Gentamycin sulfate. To investigate the effect of GCs and FF on the maturation during the rating and non-rating seasons, the oocytes co-cultured at 38.5^{0}C, 5% CO_2 and 95% relative humidity for 36 hrs **(Gordon, 1995)**. The co-culture conditions were designed as, maturation medium with no granulosa cells (MM+No GCs) (control), MM plus fresh granulosa cells (MM+FGCs), MM plus pre-incubated granulosa cells (MM+PGCs) , MM plus 10% follicular fluid (MM+10%FF) and MM+FGCs+10%FF.

The preincubation of GCs performed by placing the harvested GCs in the culture medium for 12 hrs before co-culture with oocytes.

Maturation time:

The grad 1+2 oocytes were cultured for 12 hrs, 24 hrs and 36 hrs with FGCs, to investigate the effect of culture duration on in vitro maturation of camel oocyte during rating and non-rating seasons.

Assessment of maturation :

After 12, 24 and 36 hrs of co-culture, all the expanded oocytes were agitated in 2.9% sodium citrate and cleaned in D, phosphate buffered saline to remove remained cumulus cells. All the oocytes were placed on glass slides and fixed in acetic acid and ethyle alcohol (1 : 3 v/v) overnight. The oocytes were stained with 2% aceto-orcein, and examined microscopically to assess the stage of nuclear maturation (Metaphase-I and II).

Hormonal assay:

At 24 and 36 hrs of co-culture, the media were collected, centrifuged at 3000 rpm/10 minutes and frozen at -20° until hormonal assay. Testosterone was assayed by Radio-immunoassay according to **Goetze et al. (1990)**. Progesterone was assayed according to **Naber et al. (1999)** using sold-phase ^{125}I-progesterone Radio-immunoassay (Coat-A-Count Progesterone; Diagnostic Product Corporation, Los Angeles, CA, USA). The assay sensitivity was 0.07 ng/ml (rang=0.03 to 0.16 ng/ml). The intra- and inter-assay coefficients of variation were 9.0 and 9.3%, respectively. Estradiol-17β was assayed according to **Xing et al. (1983)** by Radio-immunoassay using (Diagnostic Product Corporation, Los Angeles, CA, USA) 125I-RIA Kits. The intra- and inter-assay coefficients of variation were 9.62% and 13.43%, respectively.

Statistical analysis:

Data were expressed as the mean ± stander error. The statistical analysis of observed differences was performed by Student's t-test, and probabilities value (P) less than 0.01 was considered to be statistically significant.

RESULTS

The ovarian status of camels varied according to their reproductive season as shown in table 1. Regarding the follicular populations, the distribution of small follicles was highly significant ($P<0.01$) than the medium and preovulatory follicles either during rating or non-rating season. The medium follicles showed also higher number than preovulatory follieles during two seasons. All the follicular populations showed higher significant ($P<0.01$) numbers during rating season when compared to non-rating one.

The distribution of follicles with or without corpora lutea were significantly higher (P<0.01) during breeding season than static one, but during both seasons the number of follicles present on the ovaries was higher with than without corpora lutea. There were no significant differences in the numbers of medium and large atretic follicles when two seasons were compared, while within each season, the medium atretic follicles showed higher significant numbers (P<0.01) than large follicles.

The highest numbers of grad 1+2 oocytes were obtained during the breeding season. The mean values of two groups of ooeytes (grad 1+2 and grad 3+4) were significantly higher (P<0.01) during the breeding than non-breeding season. Although the two groups were different during the rating season, they did not show this difference during the non-rating season.

After 36 hrs of co-culture, the highest numbers of ooeytes reached metaphase-II stage of nuelear maturation was observed after the co-culture of oocytes with FGCs during both seasons (table 2). The lowest rates of metaphase-II were observed after co-culture of oocytes with PGCs.

In all co-culture groups, the rates of matured oocytes reached metaphase-II stage were higher during rating season compared to non-rating one. Although the camel oocytes matured normaly during rating season, they had potential to mature in vitro during non-rating season specially after the co-culture with FGCs or PGCs.

The overall ratios of nuclear maturation maintained higher during rating than non-rating season. The rate of nuclear maturation increased progressively with the duration of co-culture during rating season, reaching the highest at 36 hrs. The rate of nuclear maturation increased from 12 to 24 hrs, whereas it remained nearly constant up to 36 hrs during non-rating season as shown in table 3.

The steroidogenic potential of GCs varied according to the duration of in vitro co-culture (table 4). In the three groups of co-culture, the level of testosterone increased directly with increasing the duration of culture. The activity of FGCs or PGCs to secrete testosterone in culture media was significantly higher (P<0.01) during rating than non-rating season. Moreover, the highest level of testosterone observed with FGCs+FF at 36 hrs of co-culture. The same behaviour of GCs to secrete estradiol-17β observed in the first two groups (FGCs and FGCs+FF), and the highest concentrations of estradiol-17β estimated with FGCs+FF at 36 hrs of co-culture. After co-culture with PGCs, the level of estradiol-17β decreased either with increasing the duration of co-culture or from rating to non-rating season.

The highest level of progesterone obtained with increasing the duration of co-culture with PGCs. The activity of FGCs or PGCs to seerete progesterone increased progressively with the du-

ration of co-culture and from rating to non-rating season.

DISCUSSION

In spite of increased interest in the study of reproductive biology of the female camel, there has been little understanding of the relationship between ovarian morphology and the reproductive physiology of this species. In this study, the distribution of small follicles was significantly higher than medium and pre-ovulatory follicles. During rating season, different follicular populations with or without corpora lutea showed higher numbers than during non-rating season. These results come in agreement with **Musa and AbuSineina (1978) and Bourke et al. (1992)**, who observed that, camel ovaries developed a wave of multiple follicular growth during breeding season. During the rest of year the ovaries remain inactive or show only a limited number of small follicles. These encountered follicles with different sizes protruding a great part of ovarian bulk during the active season **(El-Wishy and Hemeida, 1984)**. Besides the ovulatory follicle, a few smaller follicles appeared in the same ovary, or, to a lesser extent, in the other ovary. The smaller follicles developed and regressed in variable periods but they were palpable for less time than the main follicle **(Musa and AbuSineina, 1978)**. The main follicle measured 10-15 mm at the beginning of heat but it increases to 25-30 mm at ovulation **(El-Wishy and Hemeida, 1984)**. However, the ovarian changes take place during the breeding season differ considerably from those of the cow and mare, for the camel's breeding season the activity is restricted to the follicular development only **(Musa and AbuSineina, 1978; Skidmore et al., 1996)**, and the two camel ovaries showed the follicular development throughout the whole season which ends with an anoestrous phase that lasts for about six months. The study of **Samir et al. (1978)** proved the presence of large follicles in the different seasons, and mature corpora lutea were seen only during the breeding season, whereas regressed corpora lutea were noticed all-over the year together with many follicles of different sizes. Recently formed corpora lutea during breeding season are reddish brown accompanied with presence of follicles, but firm corpora lutea occasionally seen in the ovaries of non-gravid genitalia are probably regressing corpora following infertile service **(El-Wishy and Hemeida, 1984)**. When the corpus luteum was at maximum size, there was a medium follicle on the same or the opposite ovary **(Bourke et al., 1992)**. This follicle increased in size as the corpus luteum regressed, reaching an average size of 12 mm when the corpus luteum had fully regressed. These results were interpreted by **Sghiri and Driancourt (1999)**, where during peak breeding season follicles display a high aromatase activity in their GCs than non or early breeding season.

In this work, the medium atretic follicles showed higher significant rate than large follicles in

each season, whereas there was no differences in the two types of follicular atresia when two seasons where compared. The majority of the growing follicles were found to show different stages of atresia **(Samir et al., 1978)**. A large number of mature follicles are noticed in the ovaries all-over the year. Some of these follicles are intact, others show signs of atresia. These atretic follicles appeared as either multiple whitish, pin headed solid bodies, or as dark blackish brown petichae embedded in the ovarian stroma **(El-Wishy and Hemeida, 1984)**. These results suggesting rapid follielar growth followed by rapid atresia **(Shalash, 1965)**, perhaps as a result of a commencing down regulation of LH receptors in the theca interna or GCs-layers **(Skidmore et al., 1996)**. However, a follicular wave pattern was found in camel to consist of well defined periods of growth, maturity and regression.

Although, the mean values of grad 1+2 oocytes was significantly higher than grad 3+4 oocytes only in rating season, the two groups were higher in rating than non-rating season. This observation was also obtained by **Abdoon (2001)**, where the growth and development of ovarian follicles in the camel as well as yields of oocytes with good quality were greater during the breeding than non-breeding season. These results may be attributed to the activity of camel's ovaries during breeding season **(Musa and AbuSineina, 1978; Skidmore et al., 1996)**, which is restricted only to the development of the follicular waves.

Oocyte maturation is a fundemental step and dependent upon a co-ordinated interaction between germinal and somatic cells of the follicle **(Mattioli, 1996)**. It was known that GCs from dominant follicle at least from adult animal in comparison to all other follicle sizes exhibited the highest border with gonadotropin receptors **(Khatir et al., 1997)**, and thought that cattle ooeytes are stimulated to mature in vitro through the addition of GCs from dominant follicles **(O'Doherty et al., 1996; Amer, 2000 & 2002)**. Now we have a maturation system, using a fully conditioned medium in searching for factors improving the nuclear competence acquisition to mature camel ooeytes will be possible. The present study showed that the highest rates of metaphase-II oocytes were observed after co-culture with FGCs, whereas the lowest rates were observed after co-culture with PGCs. In all groups, the rates of metaphase-II oocytes were higher during rating than non-rating season. From 30 camel oocytes cultured in vitro after removal from follicles in TCM-199, 14 were matured **(Bou, et al., 1993)**. A previous study revealed that, oocyte maturation and steroidogenesis also occur in eultured follicular cells or follicles in the absence of gonadotrophic hormones **(Readhead et al., 1979)**. Whether oocytes were co-cultured in contact or not in contact with GCs, they did not exert meiotic arrest, and the inhibitory factor(s) produced by theca cells is soluble in the medium did not exerted by GCs **(Richard and Sirard, 1996)**. Although no explanation has yet been found for different oocyte behaviours in response to use of fresh or pre-incubated GCs **(Alm, 1990)**.

The supplementation of the culture medium with GCs improves the in vitro maturation of bovine ooeytes by increasing of hormonal concentrations, which acting as paracrine factors in the culture medium that mediated by GCs **(Goetze et al., 1990; Hinrichs, 1996; Bevers et al., 1997)**, in consequence enhanees the resumption of meiosis that requires synthesis of new pre-ovulatory specific proteins (glyeosylated) mittled by GCs **(Fihri et al., 1991; Suh et al., 1993; Hashimoto et al., 1998)**. The promoting of oocytes maturation with culture GCs in vitro attributed by gradual appearence of proteins and growth hormones secreted by GCs in vitro **(Izadyar et al., 2000)**, indieate a paracrine and/or autocrine action of growth hormone on oocytes maturation. These actions also regulate several key granulosa cell enzymes involved in cumulus expansion and maintenance of an optimal oocyte microenvironment **(Elvin et al., 1999)**. Moreover, physiochemical analysis indicated that factors in GCs-cultured media were distributed in two molecular size ranges, one between 10 kDa and 30 kDa and another greater than 30 kDa **(Maeda 1996)**. These factors playing a key role in stimulating nuclear maturation appear to be epidermal growth factor secreted by GCs in vitro. Additionally, cytoplasmic maturation of the oocyte appears to be stimulated by oestradiol and epidermal growth factor, while inhibited by testosterone **(Driancourt and Thuel, 1998)**. The best of in vitro maturation was obtained at natural concentrations of estradiol produced by co-cultured GCs, whereas the worst results of in vitro maturation occurred at higher concentrations of estradiol supplementation. These data suggested an inhibitory effect of high coneentrations of estradiol on resumption of oocyte meiosis in vitro **(Mingoti et al., 1995)**. However, there was no differences between GCs collected from medium and large follieles ($1x10^6$ cell/ml) in improving maturation. This can be attributed to that GCs may contribute different components to the co-culture system based on follicle size **(Martino et al., 1993; Suh et al., 1993)**, and steroids as well as other faetors secreted by follieular cells in response to stimulation by EGF are benificial for full oocyte maturation **(Lorenzo et al., 1997)**.

In this study, FGCs and/or FF enhances maturation of camel oocytes during rating and nonrating seasons. Reeently, oocyte maturation in vitro is affected by the origin of FF which appears to vary with follicle quality but not size **(Carolan et al., 1996)**. Another studies reveaied that the size of the follicle affected oocytes maturation in vitro **(Dostal and Pavlok 1996; Wang et al., 1999)**, as FF from >15 mm follieles significantly enhances the maturation than FF from 2-8 mm follicles. No difinite effect of FF when added with GCs in culture medium, but in contrast, addition of GCs from preovulatory follicles enhanced dramatically in vitro maturation, so the preovulatory GCs environment in culture medium seems to play a major role in the further events of fertilization and embryo development **(Fihri et al. 1991)**. The data demonstrated that estradiol, progesterone and testosterone in media supplemented with FF and GCs may help to predict oo-

cyte maturity by some factors are mostly present in the healthy follicles, than regressed ones **(Enlen et al., 1998)**. It is possible that the benificial effect of FF could have been more obvious in the absence of hormones **(Sirard et al., 1998)**. Wether normal meiotic maturation of bovine oocytes is due to in part to a stimulatory factor in FF, rather than simply removal of an inhibitors was studied by **Romero et al. (1990)**. They found that after 20 hrs of incubation 40% FF stimulates a higher proportion of oocytes to reach M II than 0 hr FF and controls. Thus, the presence of maturation stimulating molecules in FF are essential for normal GCs maturation in vivo.

During rating season, the rate of nuclear maturation increased progressively with the duration of co-culture reaching the highest at 36 hrs. During non-rating season, the rate of nuclear maturation increased from 12 to 24 hrs and remained constant up to 36 hrs. These findings come in agreement with **Abdoon (2001)** who found that the maturation of camel oocytes in CRLaa medium for 36 hrs produced higher percentages of cumulus expansion and oocytes reached metaphase-II stage. The elongation the duration of maturation of camel oocytes may be accompanied with cytoplasmic and nuclear changes reaching with ooeytes to full maturation.

The stroidogentic potential of FGCs or PGCs to secrete testosterone and estradiol-17β in co-culture media was significantly increased with the duration of co-culture reaching the highest with FGCs+FF at 36 hrs, with exception the level of estradiol-17β decreased significantly after co-culture with PGCs. The potential of FGCs or PGCs to secrete progesterone increased progressively with increasing the duration of co-culturing reaching the highest level with PGC's at 36 hrs. However, the level of three hormones remained significantly higher during rating than non-rating season. These results confirmed the higher maturation rate with FGCs alone than FGCs+FF as the concentration of estradiol increased with addition of FF to culture media. This observation was confirmed by **Mingoti et al. (1995)** who obtained best maturation at natural concentrations of estradiol produced by co- cultured GCs, and an inhibitory effect of estradiol in high concentrations was exerted on resumption of oocyte meiosis in vitro. However, addition of FF to the co-culture medium potentiates the activity of GCs to secrete steroid into the medium **(Hinrichs, 1996)**, and transiently inhibit maturation. In our study, $4\text{-}5x10^6$ GCs/ml were used and produced up to 87.2% maturation rate, whereas using of $25x10^6$ and $10x10^6$ GCs/ml produced 40% and 70% maturation rates **(Hinrichs, 1996)**. In camels, the in vitro testosterone and estradiol output increased with increasing the follicular size, and was more in follicles obtained during peak breeding season than at its initiation or non-breeding season **(Sghiri and Drlancourt, 1999)**. This indicate that peak breeding season follicles display a high aromatase (the enzyme which converts androgens to estrogens) activity in their GCs than early breeding season or non-breeding season. In a previous study, progesterone and testosterone values recorded from a

control group were significantly lower than those recorded from co-culturing of oocytes with GCs ($0.5\text{-}1x10^6$ cells /ml), where 72 ng/ml and 264 pg/ml compared to 208 ng/ml and 2.168 pg/ml with fresh GCs and 364 ng/ml and 825 pg/ml with preincubated GCs **(Goetze et al., 1990)**. However, the GCs in culture without any additives maintained both estradiol and IGF-1 secretion, with basal progesterone output which suggest a role for the regulation of ovarian function **(Khalid et al., 1996)**. In rabbit, the increasing duration of co-culture GCs from 48 h to 96 h in vitro characterized by more progesterone than estradiol **(Picazo et al., 2000)**. Regarding level of progesterone, GCs cultured in vitro showed signs of luteinization as indicated by the greater release of progesterone compared to estradiol **(Hillier et al., 1984; Less et al., 1998)**. This pattern is the characteristic of GCs in vivo following the LH peak **(Mayes, 2000)**. Luteinization of GCs in vitro may be attributed to the expression of the mRNA coding for the LH receptors **(Peng et al., 1991)**. Moreover, GCs from follicles containing LH, FSH and high concentrations of estradiol underwent spontaneous mitosis in vitro and secreted significantly more progesterone than GCs from follicles which did not contain all three hormones **(McNatty and Sawers, 1975)**. This evidence was attributed to the influenee of GCs by the hormonal milieu of the follicle in vivo **(Gutierrez et al., 1996)**. In conclusion: We must evaluate the camel not only as an economic factor that could create income and employment in the national economy, but also as a country'strategie food supply in the form of meat and milk (specially for young children because it is high in water and low in fat content). Thus, more investigations are needed to establish co-culture conditions to open a way to improve the embryo production and transfer programme in Arabian camels during both rating as well as non-rating season.

Table 1: The morphological parameters of the camel ovaries in rating and non-rating (Means±S.D.).

Parameter		Reproductive season	
		Rating	Non-rating
Follicles numbers:	-small (3 - 6 mm)	18.00±1.65^{a}	8.00±1.59^{b}
	-medium (7 - 15 mm)	2.42±1.17^{c}	1.17±0.94^{bc}
	-preovulatory (>15 mm)	1.25±0.97^{ab}	0.75±0.62^{ac}
Follicles/Ovary:	-with CL	12.06±1.68^{a}	5.50±1.62^{b}
	-without CL	5.17±1.27^{b}	2.47±0.94^{a}
Atresia in:	-medium follicles	0.67±0.65^{a}	0.75±0.75^{a}
	-large follicles	0.33±0.49^{b}	0.42±0.52^{b}
Oocyte quality:	-grad 1+2	11.33±1.16^{a}	4.17±0.72^{c}
	-grad 3+4	6.17±1.53^{b}	4.33±1.23^{c}

Means±S.D with dissimilar superscripts in the same row or column are significantly different at P<0.01
CL=Corpora Lutea

Table 2: The rates of nuclear maturation of camel oocytes under different co-culture conditions for 36 hrs during rating and non-rating seasons.

Co-culture conditions of oocytes	Groups of oocytes	Stage of nuclear maturation	
		Metaphase-I n (%)	Metaphase-II n (%)
Rating season:			
- MM+No GCs	60	29 (48.30)	31 (51.70)
- MM+FGCs	86	11 (12.80)	75 (87.20)
- MM+PGCs	82	61 (74.40)	21 (25.60)
- MM+10%FF	65	29 (44.60)	36 (55.40)
- MM+FGCs+10%FF	48	23 (47.90)	25 (52.10)
Non-rating season:			
- MM+No GCs	60	39 (65.00)	21 (35.00)
- MM+FGCs	82	37 (45.10)	45 (54.90)
- MM+PGCs	80	60 (75.00)	20 (25.00)
- MM+10%FF	58	30 (51.70)	28 (48.30)
- MM+FGCs+10%FF	52	30 (57.70)	22 (42.30)

MM=Maturation Medium; GCs=Granulosa cells; FGCs=Fresh Granulosa Cells; PGCs=Preincubated Granulosa Cells; FF=Follicular Fluid

Table 3: Effect of co-culture duration on the rate of nuclear maturation (metaphase-II) of camel oocytes during the rating and non-rating seasons.

Breeding season	Duration of co-culture with FGCs		
Rating	**12 hrs (n=35)** n (%)	**24 hrs (n=40)** n (%)	**36 hrs (n=40)** n (%)
	11 (31.40)	25 (62.50)	34 (85.00)
Non-rating	**12 hrs (n=32)** n (%)	**24 hrs (n=36)** n (%)	**36 hrs (n=42)** n (%)
	6 (18.00)	18 (50.00)	22 (54.40)

Table 4: Steroids levels in culture media after maturation of camel oocytes under different co-culture conditions (Means±S.D.).

Hormone	Type of maturation media (MM)					
	MM+FGCs		MM+FGCs+10%FF		MM+PGCs	
	RS	NRS	RS	NRS	RS	NRS
Testosterone *(ng/ml)*						
24 hrs	1.44 ±0.04^{a}	1.13 ±0.05^{b}	1.74 ±0.09^{c}	1.53 ±0.08^{ab}	0.54 ±0.05^{ac}	0.39 ±0.03^{bc}
36 hrs	1.83 ±0.02^{ab}	1.38 ±0.28^{c}	2.84 ±0.07^{b}	1.79 ±0.03^{a}	0.85 ±0.03^{bc}	0.56 ±0.02^{ac}
Estradiol-17ß *(ng/ml)*						
24 hrs	390.50 ±28.86^{a}	270.60± 18.42^{b}	548.60 ±37.50^{c}	393.00 ±36.34^{ab}	199.80 ±24.71^{ac}	118.83 ±18.17^{bc}
36 hrs	574.00 ±48.45^{ab}	308.60± 52.65^{c}	855.00 ±80.55^{b}	464.33 ±51.53^{a}	118.86 ±23.91^{bc}	83.67 ±8.09^{ac}
Progesterone *(ng/ml)*						
24 hrs	110.00 ±15.74^{a}	75.83 ±8.18^{b}	115.00 ±9.22^{c}	97.83 ±14.93^{ab}	296.17 ±11.70^{ac}	123.85 ±13.19^{bc}
36 hrs	160.33 ±16.21^{ab}	99.00 ±13.27^{c}	157.33 ±9.20^{b}	110.50 ±9.31^{a}	395.20 ±32.89^{bc}	227.33 ±13.66^{ac}

Means±S.D with dissimilar superscripts in the same row or column of each hormone are significantly different at P<0.01; RS=Rating Season; NRS=Non-Rating Season.

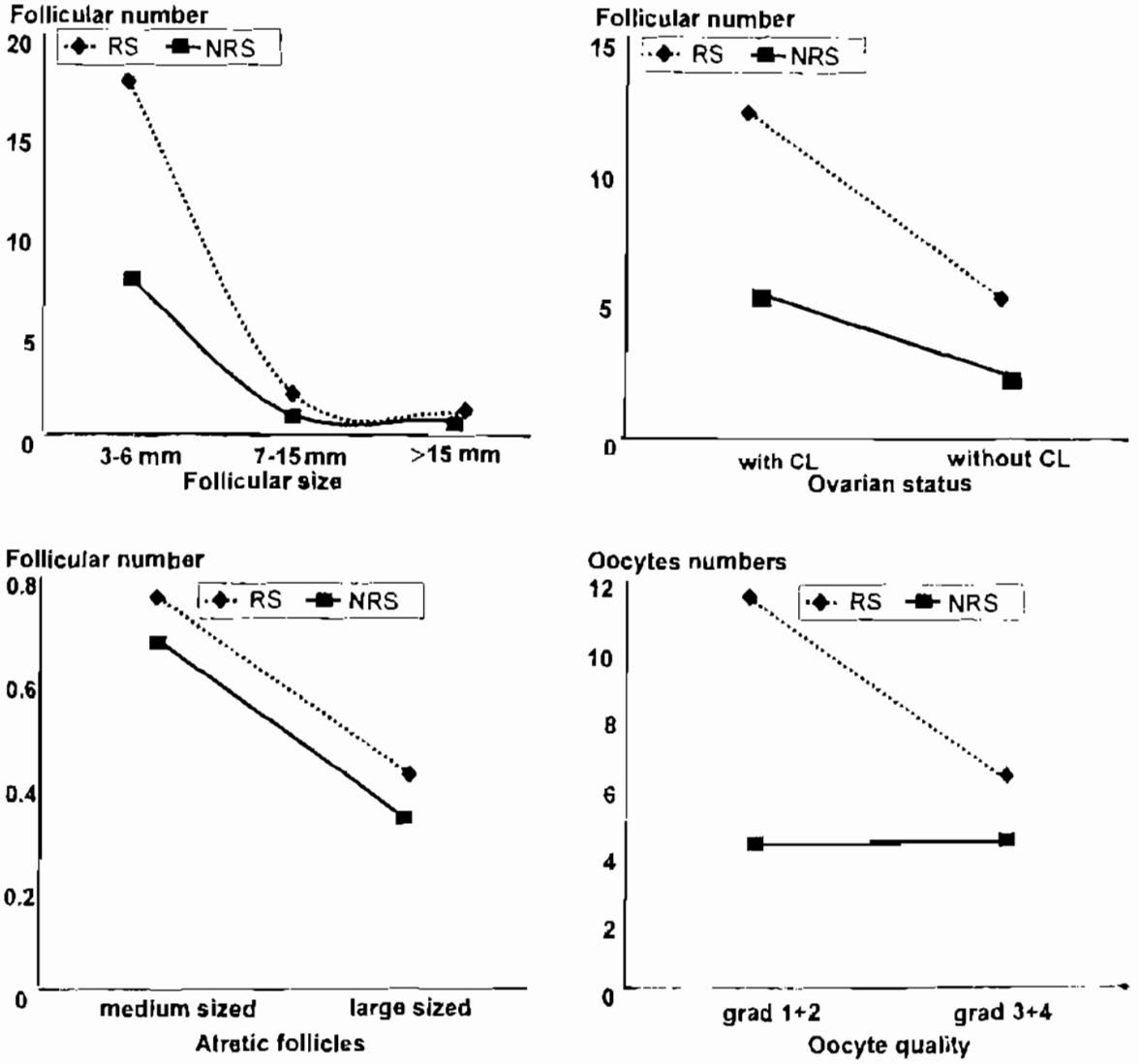

Fig.1: Reproductive status of the camel ovaries and the quality of follicular oocytes during rating and non-rating seasons.

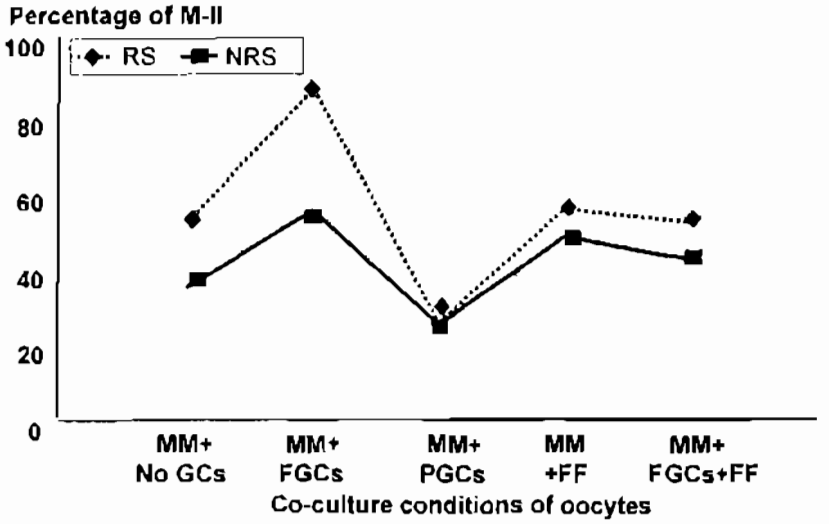

Fig.2: The rates of camel oocytes reaching metaphase-II stage of nuclear maturation after 36 hrs under co-culture conditions during rating and non-rating seasons.

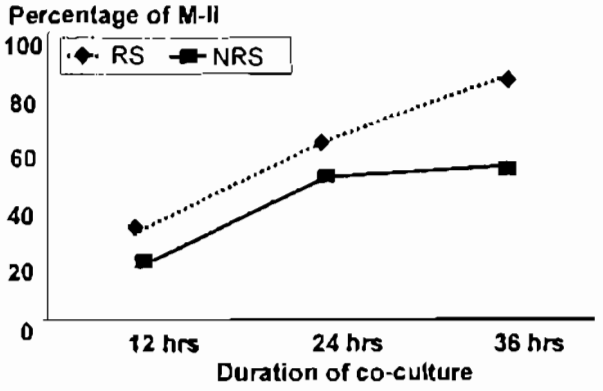

Fig.3: Percentages of oocytes reaching metaphase-II stage of nuclear maturation depending upon duration of co-culture during rating and non-rating seasons.

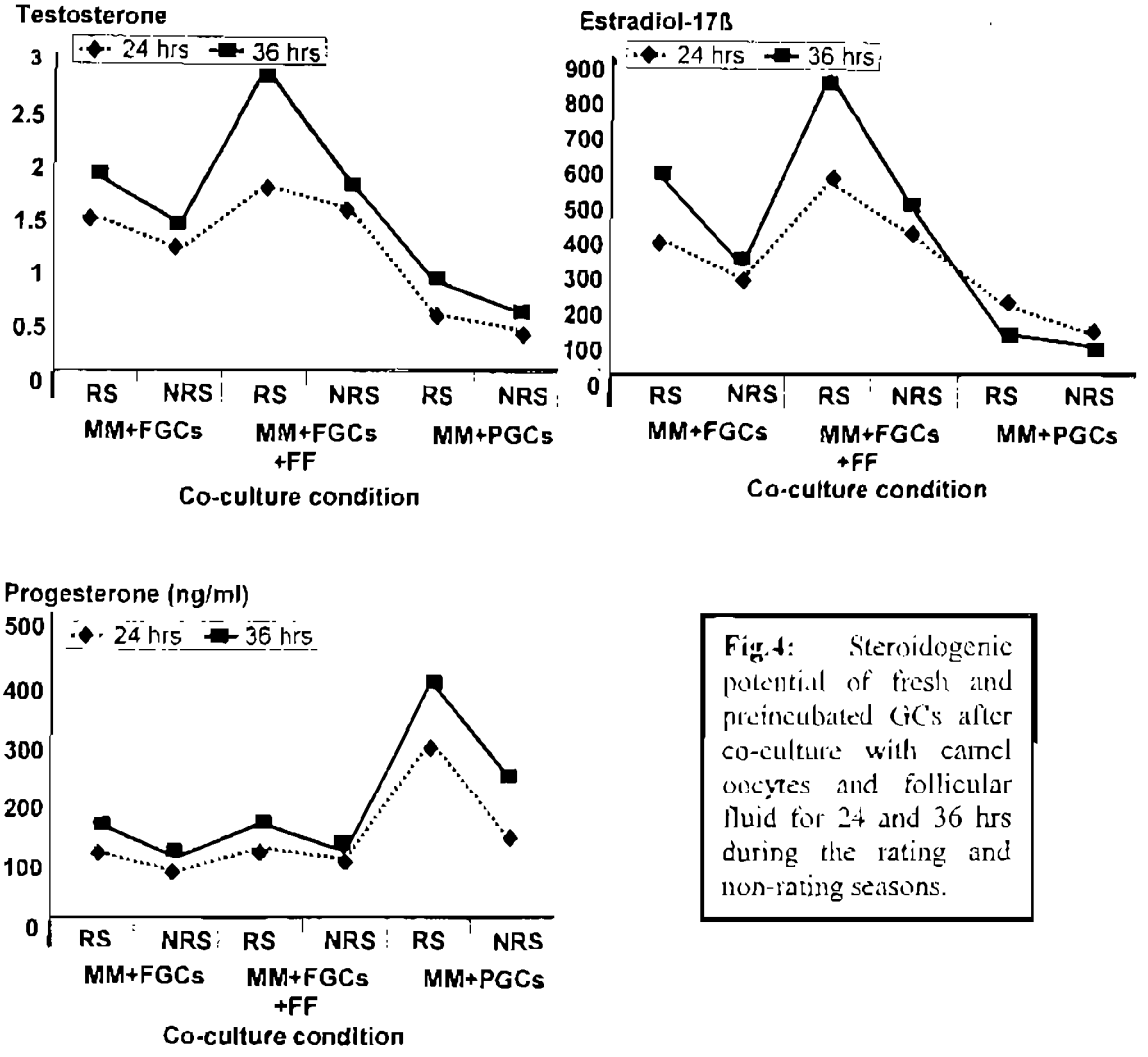

Fig.4: Steroidogenic potential of fresh and preincubated GCs after co-culture with camel oocytes and follicular fluid for 24 and 36 hrs during the rating and non-rating seasons.

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الملخص العربى

تأثير موسم التكاثر والقدرة الإستروبدوچينية للخلايا المحببة والسائل الجريبى على إنضاج بويضات الجمال معملياً

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جامعة المنصورة*، معهد الإنتاج الحيوانى - الدقى - الجيزة**.

تم دراسة تأثير حالة المبايض والقدرة الإستروبدوچينية للخلايا المحببة والسائل الجريبى على إنضاج بويضات الجمال أثناء موسمى التزاوج وخارجة. حيث تم تجميع المبايض بعد الذبح مباشرة للحصول على البويضات من الجريبات ذات الحجم ٨-٢٠مم وذلك لزراعتها فى بيئات إنضاج مختلفة مثل وسط إنضاج بدون خلايا محببة أو مضاف إليه خلايا محببة طازجة أو خلايا محببة سابقة التحضين أو ١٠٪ من السائل الجريبى أو خلايا محببة طازجة + ١٠٪ من السائل الجريبى.

وقد أوضحت النتائج أن توزيع الجريبات الصغيرة على المبايض كان ذو إرتفاع معنوى عن الجريبات متوسطة الحجم والجريبات ماقبل النضج. ،كانت جميع المجموعات الجريبية عالية معنوياً أثناء موسم التزاوج عن خارجة سراء بوجود أو عدم وجود الجسم الأصفر، بالرغم أن عدد الجريبات كان أكثر على المبايض التى تحتوى على الجسم الأصفر فى كلا الموسمين.

كما أوضحت النتائج عدم وجود إختلافات معنوية فى عدد الجريبات ذات الحجم المتوسط أو الكبير عند مقارنة الموسمين ببعضهما، بينما كان معدل الجريبات ذات الحجم المتوسط أكثر من الجريبات ذات الحجم الكبير فى كل موسم من الموسمين على حدة، ووجد أيضاً أن عدد البويضات ذات الصفات الجيدة (دوجة ١ + ٢) كان مرتفع إرتفاعاً معنوياً عن البويضات ذات الصفات الغير جيدة (درجة ٣+٤) فقط أثناء موسم التزاوج على الرغم من أن فى كلتا المجموعتين كان العدد مرتفع أثناء موسم التزاوج عن خارجه.

ووجد أيضاً أن أعلى معدل لإنضاج البويضات (M-II) لوحظت بعد إضافة الخلايا المحببة الناضجة لوسط الإنضاج وذلك أثناء موسم التزاوج، بينما لوحظ أقل معدلات الإنضاج بعد إضافة كل من الخلايا المحضنة والسائل الجريبى.

وقد لوحظ أن أعلى معدلات الانقسام الميتوزى الثانى (M-II) للبويضات قد ظهر بعد الزرع مع الخلايا المحببة الطازجة أثناء الموسمين، بينما لوحظ أقل المعدلات بعد الزرع مع الخلايا سابقة التحضين أو السائل الجريبى، وكان معدل الانقسام الميتوزى للبويضات فى كل المجموعات مرتفع أثناء موسم التزاوج عن خارجة، وأيضاً إزداد معدل إنضاج النراة مع زيادة مدة الزرع ليصل إلى أعلى معدل له عند ٣٦ ساعة أثناء موسم التزاوج، بينما ازداد هذا المعدل من ١٢-٢٤ ساعة وثبت حتى ٣٦ ساعة خارج موسم التزاوج.

كما أوضحت النتائج أن قدرة الخلايا المحببة الطازجة والمحضنة لإفراز هرمونى التيستيستيرون والاستراديول ١٧ بيتاً فى وسط الزرع إزداد زيادة معنوية بزيادة مدة الزرع ليصل إلى أعلى معدل له عند ٣٦ ساعة مع الخلايا المحببة الطازجة + السائل الجريبى، باستثناء مستوى هرمون الاستراديول - ١٧ بيتا الذى قل بعد الزرع مع الخلايا سابقة التحضين، ومن ناحية أخرى وجد أن إفراز هرمون البروجيستيرون إزداد بزيادة مدة الزرع ليصل إلى أعلى معدل له بعد ٣٦ ساعة مع الخلايا سابقة التحضين، وقد وجد أن معدل الهرمونات الثلاثة السابقة إستمر مرتفعاً أثناء موسم التزاوج عن خارجة، وفى الخلاصة نجد أن إضافة كل من الخلايا المحببة والسائل الجريبى إلى أوساط الزرع زاد من قدرة إنضاج بويضات الجمال أثناء موسم عدم التزاوج كما هو أثناء موسم التزاوج.