Global Veterinaria 13 (1): 39-46, 2014
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2014.13.01.84122

# Effect of Season and Maturation Time on Oocyte Competence and *in vitro* Maturation of Dromedary Camel Oocyte

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**Abstract:** Effect of season and maturation time on yield rate, quality and maturation rate in dromedary camel oocytes were done in 320 ovaries. The ovaries were collected from slaughter house during breeding season (December to April, n= 196) and non-breeding season (June to October, n=124).Aspiration of cumulus oocyte complex (COC) and selection of excellent and good oocytes for *in-vitro* maturation using TCM+ epidermal growth factor (EGF). Excellent and good quality oocytes recovered either in breeding or non-breeding season were cultured in maturation medium at 38.5 °C in 5 % $CO_2$ for 24 or 40 hours in humidified air. The total number of *in vitro* matured camel oocyte cultured in breeding and non-breeding season for 40 hours were 212 and 154 respectively and for 24 hours were 180 and 113 respectively. Assessment of maturation was done by detection of cytoplasmic maturation (grade of cumulus expansion) and nuclear maturation (MII) by detection of the 1st polar body. The oocyte yield rate recovered during breeding season was 10.69 (2096/196) and 6.46 (801/124) during non-breeding season. Breeding season characterize by higher percentage of excellent and good quality oocytes (74.24%, 1556/2096) when compared with non-breeding season (50.2%, 402/801) on contrary with the fair and denuded oocytes, were higher in non-breeding season (49.81%, 399/801) than breeding season (25.76 %, 540/2096). *In vitro* maturation of dromedary camel oocytes for 40 h showed higher ($P<0.01$) in cumulus expansion GIII and maturation rate (MII, $P<0.01$) either during breeding (57%, 87% respectively) or non-breeding season (48%, 37% respectively) when compared with *in vitro* maturation for 24 h were 32% and 54% respectively during breeding season and 32% and 42% respectively during non-breeding season. Maturation rate were significantly higher during breeding season either that *in vitro* matured for 40 h ($P<0.01$) or for 24 h ($P<0.05$) than during non-breeding season. In conclusion, breeding season is characterized by high number of oocyte yield rate of excellent and good oocytes quality in dromedary camel. Breeding season and maturation time have a role in cumulus expansion and maturation rate of *in vitro* matured dromedary camel oocytes. Maturation rate and GIII cumulus expansion were significantly higher during breeding season in dromedary camel. *In vitro* maturation for 40 h had high maturation rate and GIII cumulus expansion either during breeding or non-breeding season when compared with 24hrs in dromedary camel oocytes.

**Key words:** *In vitro* maturation · Season · Maturation time · Dromedary camel

## INTRODUCTION

Camel is a seasonal breeder and their reproduction is different as compared to other livestock. A rather short and limited breeding season in camel was reported between December to March in Egypt [1], Tunisia [2], Pakistan [3] and India [4], March and August in Sudan [5], October to April in Saudi Arabia [6, 7] and April to May in Somalia [8]. In Kenyan, continuous breeding was reported by Wilson [9].

Higher numbers of ovarian follicles, oocyte yield and good oocytes quality were detected during the breeding than non-breeding season in the dromedary camel [10, 11]. Oocyte quality was the most important determinant of eventual embryo yields. Presence of an intact complement of cumulus cells surrounding the oocyte and a homogenously appeared ooplasm (excellent and good quality oocytes) has been considered as the best indicators of immature oocytes ability to undergo maturation and embryonic development [12].

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*In vitro* maturation time affected the maturation rate of dromedary camel oocytes [13]. The percentage of matured oocytes (MII stage) at 30 and 40h were significantly higher than that observed at 24h and yields highest proportion of matured (metaphase-II stage) oocytes suitable for further use in assisted reproductive technologies in the dromedary camel [10, 14-18]. The period of *in vitro* culture required for nuclear maturation of oocytes is reflective for its subsequent developmental competence and most camel oocytes reached MII maturation after 36 hours of *in vitro* culture [11]. In llama, *in vitro* culture of oocytes for 36 h resulted in a significantly higher rate of maturation (MII, 62%) compared to the other incubation times [19].

There is few available literature to describe the effect of season on the *in vitro* maturation rate in dromedary camel oocytes for that, the aim of this work aimed to study 1) Effect of season on the yield rate and quality of dromedary camel oocytes. 2) Effect of season and *in vitro* maturation time in cumulus expansion and maturation rate (MII) of dromedary camel oocytes.

## MATERIALS AND METHODS

The present study was carried out in the Department of Animal Reproduction and Artificial Insemination, Veterinary Research Division, National Research Center, Cairo, Egypt. It was conducted during the period from August 2010 to May 2012.

All chemicals and media used in the present work were purchased from Sigma Company (Sant, Louis, MO, USA).

**Experiment I:** Effect of seasons on oocyte yield and quality:

Camel ovaries were collected at El-Warraq slaughter house during the breeding season (December to April, n=196) and the non-breeding season (June to October, n=124). The ovaries were placed into a thermo container containing warm normal saline solution (NSS, 0.9% Na Cl) supplemented with 100 IU/ml penicillin and 100 µg/ml streptomycin at 37°C and transported to the laboratory within 2-3 hrs. The cumulus oocytes complex (COCs) were aspirated from follicles of 2-8 mm diameter using 18 gauge needle in of aspiration medium (Phosphate buffer saline + 4mg/ml bovine serum albumin, 50ìg /ml gentamycin). During the course of study, 92 trials were preformed; the number of oocytes and recovery rate were counted under stereomicroscope (Nakamura, Japan) at 90X. The COC's were classified according to the oocyte quality to excellent and good (oocytes with 3 or more layers of complete cumulus-cells and evenly granulated dark ooplasm), fair and denuded (oocytes without or with cumulus-cells incompletely surrounding the oocyte and little granulation in ooplasm).

**Experiment II:** Effect of season and *in vitro* maturation time in cytoplasmic and nuclear maturation of dromedary camel oocytes

The collected camel COCs were washed three times with maturation medium, (TCM-199 + 20 ng/ml Epidermal growth factor (EGF, Sigma) + 10% fetal calf serum, FCS, Sigma + 50 ìg/ml gentamycin). Excellent and good quality oocytes recovered either in breeding or non-breeding season were cultured in maturation medium at 38.5 °C in 5 % CO2 for 24 or 40 hours in humidified air. These experiments were reflected for five replicates in different seasons and maturation time. The total number of *in vitro* matured camel oocyte cultured in breeding and non-breeding season for 40 hours were 212 and 154 respectively and for 24 hours were 180 and 113 respectively. Assessment of maturation was done by detection of cytoplasmic maturation (grade of cumulus expansion) i.e. G0, with no expansion; GI, with slight expansion in the outer layer of cumulus-cells; GII, with moderate expansion; GIII, with full expansion. Matured oocytes were decomulated to remove the cumulus cells by pipetting to assessment the nuclear maturation (MII) by detection of the 1st polar body.

**Statistical Analysis:** Data were expressed as percentage differences were carried out by using decision analyst STATS™ 2.0.

## RESULTS

**Effect of Seasons on Oocyte Yield and Quality in Camel:** About of 320 ovaries were collected, during breeding season (n=196) and non-breeding season (n=124). The number of aspirated oocytes/ovary (Table, 1) was higher significantly (10.69%) during breeding season (total number = 2096) than that recovered during non-breeding season (6.46 %, total number = 801). Excellent and good quality Oocytes (fig.1) showed high significance (P<0.01) during breeding season in percentage (74.24%) than in

Table 1: Effect of seasons on oocyte yield and quality in camel

| Item | Breeding season | Non-breeding season |
|---|---|---|
| No. of oocytes /ovary | 10.69**(2096/196) | 6.46(801/124) |
| Excellent and good oocytes% | 74.24 **(1556/2096) | 50.2(402/801) |
| Fair and denuded oocytes % | 25.76(540/2096) | 49.81 **(399/801) |

** Significant at P< 0.01 within the same raw

Table 2: Effect of season and maturation time in cumulus expansion percentage of *in vitro* matured camel oocytes

| | | Maturation time | |
|---|---|---|---|
| Season | Cumulus expansion | 24h | 40h |
| Breeding season | G0 | 20% (36/180)$^{**b}$ | 8% (16/212)$^{b}$ |
| | GI | 24% (43/180)$^{**}$ | 10% (22/212) |
| | GII | 24% (43/180) | 25%(54/212) |
| | GIII | 32% (58/180) | 57%(120/212)$^{**}$ |
| Non breeding season | G0 | 32% (36/113)$^{**a}$ | 14% (22/154)$^{a}$ |
| | GI | 23% (26/113)$^{**}$ | 13% (20/154) |
| | GII | 17% (19/113) | 25% (38/154) |
| | GIII | 28% (32/113) | 48% (76/154)$^{**}$ |

**P<0.01 between the raw

a-c P<0.01, a-b P<0.05 between the column

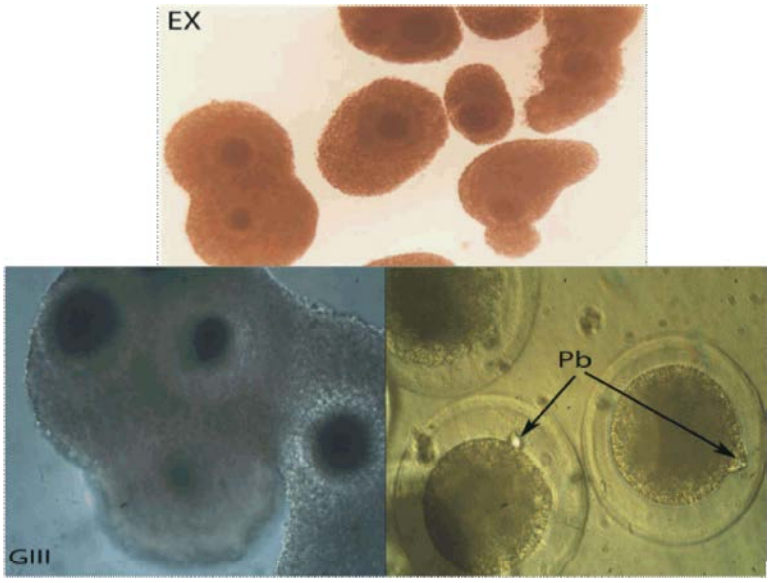


Fig. 1: Excellent (EX) oocyte quality, full cumulus expansion (GIII) and matured oocytes with 1st polar body (Pb) of *in vitro* matured dromedary camel oocytes

Table 3: Effect of season and maturation time in maturation rate (MII) of *in vitro* matured camel oocytes

| | Maturation rate (MII %) | |
|---|---|---|
| Season | 24 h | 40 h |
| Breeding season | 54% $^{a}$(98/180) | 87%$^{a**}$(184/212) |
| Non breeding season | 42%$^{b}$(48/113) | 73%$^{c**}$(112/154) |

**P<0.01 between the raw

a-c P<0.01, a-b P<0.05 between the column

non-breeding season (50.2 %). Non-breeding season showed higher significant (P<0.01) values in low quality oocytes (fair and denuded) in percentage (49.81% respectively) than breeding season (25.76 %).

**Effect of Season and Maturation Time in Cumulus Expansion Percentage and Maturation Rate (MII) of *in Vitro* Matured Camel Oocytes:** Cumulus expansion GIII (Fig. 1) were highly significant (P<0.01) in *in-vitro* matured camel oocytes cultured for 40 hours (Table 2) during breeding season (57%) and non-breeding season (48%) when compared with 24 hours (32 and 17 % respectively). Camel oocytes *in vitro* matured for 24 hours (h) showed high significance (P<0.01) no (G0) and slight (GI) cumulus expansion percent (36 and 43 respectively) when compared with that *in vitro* matured for 40 h (8 and 10 respectively) in breeding season. Moreover, during non-breeding season cumulus expansion were significantly higher (P<0.01), 36 % (G0) and 26% (GI) in 24 h than 14 % (G0) and 13% (GI) in 40 h. Non breeding season was

characterized with higher percentage of no cumulus expansion (G0) than breeding season either for camel oocytes *in vitro* matured for 24 or 40 hours.

Maturation rate (MII, Fig. 1, Table 3) were significantly higher (P<0.01) in camel oocytes *in vitro* matured for 40 h (87 and 73) when compared with 24 h *in vitro* matured (54 and 42) either during breeding or non-breeding season respectively. Maturation rate of *in vitro* matured camel oocytes either for 24 h (P<0.05) or 40 h (P<0.01) were significantly higher during breeding season than non-breeding season.

## DISCUSSION

This work revealed that camel ovarian follicles are continuing to grow and develop during the breeding and non-breeding seasons in the dromedary camel. However, their number was affected by the season of the year. Higher significant (P<0.01) number of aspirated oocyte/ovary were detected during the breeding season where the mean of total number of oocytes recovered per ovary was 10.69 while it was 6.46 during non-breeding season.There were seasonal differences in ovarian activity in camel [20] and the mean number of oocyte recovered per ovary were higher during the breeding season than non-breeding season [10,14]. Seasonal effect on camel ovary to a higher incidence of incomplete follicular waves, in which the growing follicles was failed to reach maturity during non-breeding season [21]. Follicular maturation in camel ovaries was affected by season, as follicular growth during the seasonal transition period was associated with a reduced oestradiol, despite a similar supply of androgen to that observed during breeding season [20]. This indicated that aromatase activity of granulosa cells was reduced during non-breeding season. Ovaries of camels remained inactive during the non-breeding season or had only a limited number of very small follicles [22]. This discrepancy may be due to the effect of different environmental and nutritional factors, to latitude differences or to technique differences [5]. The result of this work showed average number of oocytes (10.69 and 6.46) during breeding and non-breeding season. In the literature there are wide variations in number of oocytes per camel ovary, being 3.99 [23,24], 4.1 [25,26], 5.3 [27], 6.67 (28), 7.64 (10) and 12.4 (29). The high number of oocytes/ovary obtained in this study as compared to the others previously reported on camel may be attributed to pronounced differences in animal ages, seasonal differences [5,10,20], reproductive status [10,14], site of the ovary [24,11] and method of oocytes collection [30,31,32,33]. Follicular growth and maturation were optimal during the breeding season. Follicles continued to grow in a lesser number during non-breeding season but failed to reach maturity as indicated by the presence of a fewer medium and large sized follicle. Accordingly the results of the present study showed that the percentage of excellent and good quality oocytes was (74.24%) that was significantly higher (P<0.01) during breeding season than during non-breeding season (50.2%). While, percentage of fair and denuded oocytes collected during non-breeding season was significantly higher (P<0.01) than those collected during breeding season. In agreement with the present results, the total number of excellent and good quality oocytes was significantly higher during breeding season compared with non-breeding season [11]. This difference may be associated with the presence of more follicles during the breeding season. Moreover, the good quality oocytes during breeding season may be due to the cold weather and the good nutrition that reflect on the oocyte quality [10]. Presence of an intact complement of cumulus cells surrounding the oocytes and a homogenously appeared ooplasm has been considered as the best indicators of an immature oocytes ability to undergo maturation and embryonic development. In addition the cumulus investment morphology and the microscopic aspect of the ooplasm are generally considered as the two main parameters to assess the quality of the cumulus oocytes complexes (COCs) [34, 35]. The existence of a healthy population of somatic cells surrounding the oocytes is mandatory to facilitate the transport of nutrient and signals into and out of the oocytes [36]. On the other hand, oocytes with expanded cumulus cells complex and irregular ooplasm exhibit a decreased capacity to mature *in vitro* [37]. Also, oocytes that are not surrounded by a tight and complete multi-layered cumulus investment and contained an ooplasm with a sandy appearance have aberrant protein synthesis patterns [38].

The present study showed that the *in vitro* maturation rate were highly significant during breeding season in comparison with non-breeding season, at 40hrs and 24 hrs (87% and 54%vs. 73% and 42% respectively), this in agreement with the fact that addition of FSH or eCG hormone to maturation media increase the maturation rate of camel oocytes during breeding season in comparison with non-breeding season [11]. Unfortunately, one literature concerning the effect of season on maturation rate of camel oocytes [11] or other mammalian animal except one study that had been done in sheep [39] that no difference in efficiency of *in vitro* maturation oocytes

collected during breeding and non-breeding season. They also added that the presence of good number of antral follicles on the ovaries of sheep is frequently observed even during the non-breeding season. The higher number of oocytes / ovary obtained in this study during breeding season associated with higher mean number of excellent and good number quality oocytes during breeding season all this findings may be the cause of increased maturation rate that observed with the present study. Thus seasonal effect may induce complete follicular waves in which the growing follicles successes to reach maturity during breeding season. The effect of different environmental and nutritional factors or techniques used may play a role in improving oocyte quality that reflects in increasing the *in vitro* maturation rate of oocytes [20].

The degree of cumulus cell expansion and meiotic maturation rate had a relative importance in evaluating *in vitro* maturation. The induction of maturation in oocytes depends on the completion and integration of a number of essential processes associates with both the nuclear and cytoplasmic components of maturation [40]. The results in this study showed that the cumulus expansion GIII (cytoplasmic maturation) and maturation rate (nuclear maturation) were highly significant ($P< 0.01$) in 40hrs maturation time when comparing with 24hrs maturation time during breeding season (57% vs. 32% and 87% vs. 54% respectively) and during non-breeding season (48% vs. 28% and 73% vs. 42% respectively). In contrary, the cumulus expansion G0 and GII were significantly higher in camel oocytes matured for 24 h than 40 h either in breeding or non-breeding season. The results are in agreement with previous works, higher ($P<0.01$) cumulus expansion and maturation rate of camel oocytes matured in CR1aa, ranging between 38.8 and 85.4% by increasing maturation time from 24h to 36h [10], camel oocytes matured in TCM199 for 30 h showed 58% maturation rate [14]. Moreover, 40-44 h of *in vitro* maturation, yields highest proportion of matured (metaphase-II stage) oocytes suitable for further use in assisted reproductive technologies in the dromedary camel and the highest proportion of M-II oocytes (52%) was obtained at 44h when compared to 40 h of *in vitro* maturation (15). Proportions of oocytes reaching M-II stage at 32h (42%), 36h (45%), 40h (49%,), 44h (52%,) and 48 h (46%,) at TCM199 [16]. *In vitro* culture required for nuclear maturation of oocytes is reflective for its subsequent developmental competence and most camel oocytes reached MII maturation after 36 hours of *in vitro* culture when the media is supplemented with FSH or eCG [11]. Camel oocytes reached the M II stage (66%) at 30 h in using Ham's F10 medium [13] and 44.06% maturation rate of *in vitro* matured camel oocytes for 30 h [27]. The differences observed by authors in maturation rates recorded in the maturation time may be due to some factors like seasonal variation [11], hormones [41,17], maturation media [42], addition of Epidermal growth factor [24,43] and maturation period [10,23,44,17]. Camel oocytes maturation improved by increasing time of *in vitro* culture may be this time for cytoplasmic maturation associated with reorganization of cytoplasmic organelles [45]. Mammalian oocytes maturation and cumulus expansion are hormone induced simultaneous processes that involve changes in cumulus-cell shape and in the interactions between cumulus cells and the oocytes [46]. The cumulus oopherus expands after ovulation due to the deposition of a proteoglycan matrix, the major carbohydrate in this mucoelastic matrix is hyaluronic acid [47]. The importance of cumulus cells are for cytoplasmic and/or nuclear maturation of buffalo oocytes [48, 49]. Cumulus cell (CC) is connected with oocytes by gap-junctions that allow small molecules and information exchange between cells [50]. In buffalo, cumulus cells expansion is routinely employed in IVM for evaluating oocytes maturation, but no information is available on its relation with the occurrence of nuclear maturation. The degree of expansion of cumulus cell mass is routinely considered in evaluating oocytes maturation in buffaloes [51, 42]. The oocytes with degree 2 and 3 cumulus cell expansion and extruded first polar body as matured and can be used for *in vitro* fertilization [49, 52]. The granulosa cells supply the oocyte with nutrient and connect them to the external world [53]. Cumulus cells offer nutrients to oocytes, participate in the resumption of meiosis and promote ooplasm maturation during oocytes maturation [54, 55]. Metaphase II, two groups of unequally spread chromosomes were observed and the polar body set was clustered together [56] occurred in different steps according to maturation time [57].

In conclusion, breeding season is characterized by high number of oocyte yield rate of excellent and good oocytes quality in dromedary camel. Breeding season and maturation time have a role in cumulus expansion and maturation rate of *in vitro* matured dromedary camel oocytes. Maturation rate and GIII cumulus expansion were significantly higher during breeding season in dromedary camel. *In vitro* maturation for 40 h had high maturation rate and GIII cumulus expansion either during breeding or non-breeding season when compared with 24hrs in dromedary camel oocytes.

**ACKNOWLEDGMENT**

This work was financially supported by Academy of science technology of Egypt, through EMHOTP program in project: Effect of Heat Stress on Early Reproduction Success in Cattle and Buffalo (French-Egypt joint project ID: EGY-FR/9-003,118, 2012-2014). I would like to thanks my French partner Prof. Dr. Pascal Mermillod, who supplied this work with epidermal growth factor.

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