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Original Article *Theriogenology*

The effect of season and ovarian storage time on oocytes recovery and quality in dromedary camel

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ABSTRACT

Objective: The current study aimed to investigate the impact of season (in- and out of the breeding season), and ovarian storage time on oocyte quality and IVM rates.
Design: Randomized controlled experimental study
Animal: Camel ovaries were collected from El-Bassatin abattoir located in Cairo, Egypt during the period from October 2016 to July 2018.
Procedures and results: To investigate the impact of seasonal variations on oocyte recovery and quality, ovaries were collected (in- and out of the breeding season), and the recovered oocytes were graded (I, II, III, and IV). Higher percentage (P <0.05) of grade I & II oocytes was recovered in the breeding season group when compared to out of the breeding season group (74.76±4.69% vs. 42.40±9.96%, respectively). To investigate the effect of storage time on oocyte recovery, quality and IVM rate, ovaries were collected during the breeding season and then stored at 4 °C for up to 0-2, 2-4, 4-6, or 6-8 h. The highest recovery rate (P <0.05) of grade I & II oocytes was achieved in the first group (up to 2 h; 78.21±11.09%) and this rate declined with the advancement of storage time.
Conclusion and clinical relevance: In summary, the yields of grade I & II oocytes were greater in the breeding season in comparison to out of the breeding season. Moreover, the increase of time interval between animal evisceration and ovaries processing to obtain oocytes (storage time) has negatively affected oocyte quality.

Keywords: Camel, Oocyte, Season, Quality, Recovery rate.

1. INTRODUCTION

Camels are seasonal breeder animals and their reproductive physiology differ from that of other livestock in that during breeding season, both male and female enter into sexual excitement. The expression used in case of males is “Thoot”, “Rutt” or “Musth”. Usually, the heat period is from November to March. The she camel matures at 3-4 years old whereas males at 4-5 years old [1-3].

The reproductive performance of camels under natural conditions is relatively lower than other species [4]. The reason for that might be attributed to the relatively short breeding season, longer prepubertal period, long gestation period (13 months), and prolonged (8–10 months) lactation period-related anestrus leading to a long inter-calving interval [4].

The utilization of assisted reproductive technologies (ARTs) could be essential for profitable production in camelids. The mammalian oocytes are considered the corner stone for emergence of new ARTs like *in vitro* embryo production (IVP), intracytoplasmic sperm injection (ICSI), cloning as well as stem cell approach [5]. Consequently, a variety of techniques has been established to get the mammalian oocytes. For instance, ultrasound-guided ovum pick-up (OPU) technique and ovariectomy surgery have been used to get oocytes from the live animals. On the other hand, the slaughterhouse ovaries are a plentiful source for the mammalian oocytes [6]. Due to the slower advancement of IVP protocol in camelids in comparison with the other species. The *in vitro* embryo developmental competence largely depends on the oocyte quality and successful achievement of oocyte *in vitro* maturation (IVM) process [7]. Therefore, the current study aimed to study the impact of season (i.e., in breeding season and out of breeding season), and ovaries storage time on oocyte quality and IVM rates.

2. MATERIALS AND METHODS

The current study was conducted at Department of Theriogenology, Faculty of Veterinary Medicine, Mansoura University in association with Artificial Insemination and Embryo Transfer Department, Animal Reproduction Research Institute (ARRI), Al-Haram, Giza, Egypt, during the period from October 2016 to July 2018.

2.1. Reagents and Media

All reagents and media, otherwise mentioned, were obtained from Sigma (St Louis, MO, USA).

2.2. Oocyte collection

Camel ovaries were collected from El-Bassatin abattoir located in Cairo, Egypt during the period from October 2016 to July 2018, and transported within 2 hours (h) to the laboratory in a warm 0.9% NaCl solution supplemented with 100 mg/ml streptomycin and 100 unit/ml penicillin. Using an 18-gauge needle attached to a 10 ml syringe, the follicular fluid was aspirated from follicles (3-8 mm in diameter) and put in conical tubes containing a modified phosphate buffer saline (mPBS) with 10% fetal calf serum (FCS; Gibco®, Thermo Fisher Scientific®). After aspiration accomplishment, the tubes were kept for 15 min at 39 ºC for allowing the cumulus oocyte complexes (COCs) to settle down as previously described [8].

Using a plastic Pasteur pipet, the pellet was collected from the bottom of each tube and then poured in a sterile 60 mm petri dish for COCs searching under stereomicroscope; only oocytes with multiple layers of compact cumulus cells and homogenous ooplasm were selected [9].

The recovered COCs were classified into four grades as follows: grade I (good quality) which includes the oocytes covered with more than three layers of cumulus cells, grade II (fair quality) which includes the oocytes covered with one to three layers of cumulus cells, grade III (poor quality) which includes denuded oocytes. All the former three grades, ooplam is homogenous while grade IV oocytes includes oocytes with uneven dark ooplasm [10]. In the present study, we utilized only grade I & II COCs.

2.3. Oocyte in vitro maturation

The collected oocytes were washed three times TCM-199 (with Hank's salts without phenol red and supplemented with 10% FCS) and then incubated (groups of 10-15 COCs) in 50 µL of pre-warmed TCM-199 medium (with Earle's salts and supplemented with 10% FCS, 10 µg/ml FSH, 50 mg/ml sodium pyruvate and 50 µg/ml gentamycin covered with a sterile mineral oil for 30 h at 39 ºC and 5% CO_2 (control group).

At the end of maturation period, the oocytes were inspected for the maturation signs based on nuclear maturation after staining with aceto-orcein as defined earlier [11]. In brief, COCs were subjected to gentle pipetting for cumulus cells removal and then transferred to 1% hypotonic solution of sodium citrate for 3 min. Groups of 10-15 oocytes were placed on a clean glass slide and covered with a glass cover slip secured by four spots of vaseline on the four corners of the slide. While observing the oocytes under the stereomicroscope, a slight pressure was done to the corners of the cover slip till a small vacuole appears around the oocytes. The slides were then put in a jar containing a fixative solution ethanol: acetic acid (3:1) for at least 24 h. After fixation, the oocytes were stained by 1% aceto-orcein stain, then inspected under a phase contrast microscope to evaluate the nuclear maturation status based on nuclear chromatin configuration. Oocytes at metaphase II (MII) stage were recorded as mature ones [12].

2.4. Experimental design

Experiment 1. Effect of seasonal variation on the oocyte quality:

The retrieved oocytes were divided into two groups as follows; group 1 contains oocytes collected in the period from October to April (in breeding season oocytes), while group 2 contains oocytes collected in the period from May to July (out of breeding season oocytes).

Experiment 2. Effect of time after slaughter on the recovery rate and oocyte quality

The retrieved oocytes were divided into four groups ovaries as follows: group 1 contains oocytes collected from ovaries up to 2 h after slaughter, group 2 contains oocytes collected from ovaries up to 2-4 h after slaughter, group 3 contains oocytes collected from ovaries up to 4-6 h after slaughter, while group 4 contains oocytes collected from ovaries up to 6-8 h after slaughter,

2.5. Statistical analysis

Data are presented as Mean ± standard error of mean (SEM), unless otherwise stated. At least, four replicates were carried out for each experimental group. Data were analyzed using Chi-square test [13] and $P < 0.05$ was considered significant.

3. RESULTS

2.6. Effect of seasonal variation on the recovery rate and oocyte quality

Our data demonstrated that the camel ovaries collected in breeding season are associated with higher percentage (P <0.05) of grade I & II COCs in comparison with those collected out of breeding season (74.76±4.69% vs. 42.40±9.96%). On the other hand, our study revealed higher percentage (P <0.05) of grade III & IV COCs in ovaries collected out of breeding season in comparison those collected in breeding season (33.78±4.52% vs. 14.66±2.26%) and (23.81±8.18% vs. 10.58±2.50%), respectively as shown in

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2.7. Effect of time after slaughter on the recovery rate and oocyte quality:

Our findings revealed that the highest percentage (P <0.05) for the recovery of grade I & II COCs was at 0-2 h after slaughter (78.21±11.09%) and decreased with time advancement to be the lowest at 6-8 h after slaughter (45.09±3.67%). In reverse, the longer the time interval was, the higher the percentage of grade III & IV COCs was as shown in

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4. DISCUSSION

Due to its capacity to produce good-quality meat, milk, and fibers in tough environmental conditions, the dromedary camel is a highly important species. Besides, the dromedary camels could be used in agriculture, racing as well as transportation. As a result, millions of people living in water-scarce areas mainly depend on the dromedary camels for their economic well-being [4].

Table 1: Effect of seasonal variation on the recovery rate and oocyte quality

Breeding Season	No. of ovaries	No. of oocytes (Oocytes/ovary)	Oocyte quality		
			Grade I & II	Grade III	Grade IV
In	105	483 (4.59±0.62) [a]	357 (74.76±4.69%)[a]	73 (14.66±2.26%)[b]	53 (10.58±2.50%)[a]
Out	76	137 (1.82±0.63) [b]	49 (42.40±9.96%)[b]	40 (33.78±4.52%)[b]	48 (23.81±8.18%)[b]

Values with dissimilar superscripts in the same column are significantly different at P <0.05.

Table 2: Effect of time after slaughter on recovery rate and oocyte quality

Time	No. of ovaries	No. of oocytes (oocytes /ovary)	Oocyte quality		
			Grade I & II	Grade III	Grade IV
0-2 h	67	288 (4.35±0.35)[b]	224 (78.21±11.09%)[a]	37 (12.68±6.45%)[a]	27 (9.11±4.75%)[b]
2-4 h	95	42 (4.53±0.41)[b]	299 (70.86±3.79%)[ab]	78 (17.66±2.61%)[a]	50 (11.47±1.64%)[b]
4-6 h	123	639 (5.24±0.62)[ab]	333 (53.08±4.33%)[bc]	143 (22.26±4.86%)[a]	163 (24.65±4.6%)[a]
6-8 h	154	955 (6.19±0.09)[a]	431 (45.09±3.67%)[c]	214 (22.80±2.49%)[a]	310 (32.10±3.15%)[a]

Values with dissimilar superscripts in the same column are significantly different at P <0.05.

4.1 . Effect of seasonal variation on the recovery rate and oocyte quality:

The dromedary camels are seasonal breeder animals; their pretty short breeding season occurs in winter months when the ambient temperatures become lower and grass conditions become better [2]. In Egypt, the breeding season of dromedary camels extends from December to April [1].

In the present study, the ovaries collected in the breeding season recovered a greater proportion of grade I & II COCs. On the contrary, the ovaries collected out of the breeding season recovered a greater proportion of grade III & IV COCs. These findings could be attributed to the fact that out of the ovaries out of breeding season are dormant with defective follicular growth and maturation [14]. However, some Egyptian studies reported that the dromedary she-camel can conceive during the whole year regardless the occurrence of breeding season [15] .

In line with our results, some previous studies investigated the effect of season on follicular growth and maturation in camels; they found that the percentage of she-camels with active ovaries (i.e., ovaries showing follicles >5 mm) increases from 73.5% in October to December to become 89.0% in January to May [16]. In addition, the quantity and quality of collected oocytes significantly varies over the year. In other words, the camel ovaries collected in breeding season yielded 6.73 COCs/ovary among them 5.93 COCs of grade I, while those collected out of breeding season yielded 5.40 COCs among them 4.70 COCs of grade I [17].

In such manner, the higher environmental temperatures are associated with lower oocyte recovery rates. Definitely, the recovery rate of ovaries collected in August was a much lower than those collected during the rest of year (0.78 COCs/ovary vs. 1.6-3.0 COCs/ovary, respectively) [12]. Besides, the quality of collected oocytes is negatively impacted during the summer months. Precisely, the recovery rate of grade I

COCs was the highest during December, January, and February (32.1%, 36.0%, and 38.4%, respectively) when compared to the other months (10.4%-18.3%) [12]. As a result, the percentage of oocytes selected for IVM procedures was high (100%) during November-February, tended to decrease during August-October (92.2% to 93.2%), and recorded to be the lowest (75.0% to 89.6%) during March-July. Moreover, the oocytes collected in July showed a higher percentage of cellular degeneration [12].

Also, an earlier work clarified the difference in IVM rates in relation to the period of oocyte collection and showed a significant decrease in IVM rates of the oocytes collected during April-August (30.0-41.3%) when compared with those collected during September-March (47.7%-58.7%) [12]. Altogether, it is clear that the breeding season has a great impact on the quantity and quality of recovered oocytes which affects the success of IVM process in dromedary camel.

4.2 Effect of time after slaughter on recovery rate and oocyte quality

Nowadays, the slaughterhouse ovaries are the predominant source of oocytes used for IVP in most domestic animals. Oocyte quality, in terms of cytoplasmic and nuclear maturation and developmental competence after *in vitro* fertilization (IVF), could be harmed by the long transport from the slaughterhouse to the laboratory before start of IVM process [18-22]**.**

Indeed, the existing work investigated the effect of time elapsed from the ovarian collection to oocyte recovery. Importantly, our data revealed that the earlier collection time post-slaughter was accompanied by higher proportion of grade I & II COCs and lower proportion of grade III & IV COCs than the later post-slaughter time, and vice versa.

In such manner, a previous study reported no significant difference in the recovery rates of oocytes collected from ovaries stored at 25-30 ^{o}C for 4 h when compared to those stored for 0-30 min. However, the latter oocytes were accompanied by higher IVM rates [12]. However, another work showed that oocyte recovery rate and quality are negatively affected with time passing post-slaughter. To clarify, the camel ovaries stored for 5 h post-slaughter yielded 12.4 oocytes/ovary, and then the yield declined to be 7.1 oocytes/ovary by increasing the time post-slaughter to 24 h [23]. Moreover, the camel ovaries stored for 5 h post-slaughter showed the highest percentage of grade I oocytes (65%) which gradually decreased to be 34% at 24 h [23]. One limitation in such study is that the shortest time interval was 5 h which is longer than our study.

On the other hand, parallel studies were conducted in the other domestic animals. To begin, it has been demonstrated that the bovine ovarian storage for 8 h at 37 ^{o}C significantly reduced oocyte developmental competence [19] and similar results were obtained when the ovaries were stored at 4^{o} C for 12 h or 24 h [20]. Likewise, it has been shown that the bubaline ovarian storage at 4^{o} C for 4-8 h significantly reduced the oocytes quality [24].

In regards to the ovine oocytes, it has been found that the maturation rates declined over the time after storage at 4 ^{o}C, 22 ^{o}C or 37 ^{o}C, but the oocytes from ovaries stored at 22 ^{o}C showed higher maturation rates in comparison with the others [18]. On the contrary, it has been reported that the storage of equine ovaries at 27-37 ^{o}C for 6-8 h did not greatly affect the cytoplasmic as well as nuclear maturation of the oocytes [25]. Nonetheless, another study revealed that the oocytes recovered from equine ovaries stored at room temperature for 15-18 h had lower maturation rates (27%) than those immediately recovered post-slaughter (72%) [26].

In a similar way, it has been recorded that the storage of porcine ovaries at 35 ^{o}C for either 6, 9, or 12 h decreased IVM rates and increased the propagation of DNA damage in the recovered oocytes [21]. However, a recent study has shown that a 2 h transportation time of the porcine ovaries at 25-35 ^{o}C kept satisfactory oocyte quality, maturation and fertilization rates [22]. Therefore, it seems that a much longer time passed between the slaughter and oocyte recovery has a negative impact on the oocyte quality and the subsequent IVM rates in dromedary camels.

Conclusion

In summary, the yields of grade I & II oocytes were greater in the breeding season in comparison to out of the breeding season. Moreover, the increase of time interval between animal evisceration and ovaries processing to obtain oocytes (storage time) has negatively affected oocyte quality.

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