



Effect of Heat Stress on Developmental Competence of *In Vitro* Matured Oocytes of *Camelus Dromedaries* with Different Qualities

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ABSTRACT

The deleterious effect of heat stress on cumulus-oocytes complexes (COCs) competence is well recognized in different livestock species. Therefore, the present study aimed to investigate the effect of physiologically relevant heat stress on the developmental competence of camel COCs during *in vitro* maturation (IVM). A total of 1548 COCs were divided into six groups in this study. The groups were named K1 and K2 representing good and low-quality COCs incubated at 38.5°C for 30 hours. While K3 and k4 represent good and low-quality COCs exposed to 41°C for the first 6 hours of IVM. Finally, K5 and k6 represent the groups of good and low-quality COCs exposed to 42°C for the first 6 hours of IVM. After exposure of COCs to heat stress at 41°C and 42°C during the first 6 hours of *in vitro* maturation, the COCs were incubated at 38.5°C for 24 hours of IVM. The *in vitro* matured COCs were activated to cleave using ethanol followed by 4 mM 6-DMAP and developed embryos were cultured *in vitro* for 7 days post parthenogenetic activation. The results of this study indicated that heat stress at 42°C significantly decreased the Pb (polar body) extrusion rate in K4 and K6, compared to other groups. Additionally, the embryo cleavage rate was significantly lower for good and low-quality oocytes exposed to heat stress (K2, K3, K4, K5, and K6), compared to good quality COCs of the control group (K1). The cleavage rate was lower for low quality (K2; 63 ± 1.28) than good quality COCs (K1; 53 ± 1.85). The percentages of oocytes that developed to the blastocyst stage were lower for K2, K3, K4, K5, and K6 than K1. Moreover, the blastocyst rate was lower for K2 (9 ± 0.22) than K1 (15 ± 0.22). The results of this study indicated that exposure of camel oocytes to heat stress for 6 hours during *in vitro* maturation severely reduced extrusion of polar body, cleavage, and blastocyst rates. The low-quality camel COCs were reduced developmental capacity than good quality oocytes.

Keywords: Camel, Embryo development, Heat stress, Oocyte

INTRODUCTION

Nowadays, there has been concerted attention to global warming, especially in tropical and subtropical regions, due to the increased level of average air temperature during recent years (Rabie, 2020). Egypt is located in a subtropical climatic region, which encourages the adaptation to the extreme heat stress and drought of the desert. In this regard, the Arabian camel, known as *Camelus dromedaries*, has developed unique anatomical as well as physiological and biochemical characteristics (Hoter et al., 2019) to cope with such challenges. *Camelus dromedarius* is a source for meat, wool, and milk in desert areas heavily affected by the heat and shortage of feed and water (Yaqoob et al., 2017).

Camels' population in Egypt consists of 148060 animals contributing to approximately 36398 tons of meat (i.e., 1.7 of total meat production in Egypt, (FAO, 2015). In response to climatic change progress, there is a promising role for camels due to their high resistance to heat and arid conditions. The reproductive performance of *Camelus dromedaries* is considered one of the most important factors affecting camel productivity (Tibary and El Allali, 2020). Heat stress has negative effects on the reproductive efficiency of dairy cows. It has been reported that heat stress could increase ovarian inactivity, which is manifested by anestrus (Wolfenson et al., 2000; Oseni et al., 2003). The reduction in estradiol biosynthesis is the mechanism by which heat stress deteriorates ovarian follicle development and impaired expression of estrus (Badinga et al., 1993; Wolfenson et al., 1988). The ovarian follicles and their enclosed oocytes are highly sensitive to hyperthermia (Roth, 2017; Islam et al., 2018). (Camargo et al., 2019) stated that heat shock increases the proportion of apoptotic oocytes during *in vitro* maturation (Camargo et al., 2019). The findings of a recent study suggest that camel oocytes are less tolerant of the short acute heat shock, which is linked with reduced ooplasm diameter and maturation rate as well as increased incidence of chromosomal count abnormalities (Islam et al., 2018). In fact, the slow development of assisted reproductive technologies, such as Artificial Insemination (AI), embryo transfer, and IVEP, represents a major obstacle in the wide application of these techniques in *Camelus dromedaries* (Abdoon et al., 2014). P

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Parthenogenetic activation of the metaphase II oocytes in such species as camelids provides the chance to evaluate the development ability of oocytes without the need to an external factor (sperms). Therefore, the present study was carried out to examine the effect of heat stress during *in vitro* maturation on the developmental competence of camel oocytes with different morphological qualities up to the blastocyst stage after parthenogenetic activation.

MATERIALS AND METHODS

This study was carried out at the Central Laboratory for Research, MSA University, Egypt. All the employed reagents and media used in the current study were obtained from Sigma–Aldrich (St. Louis, MO, USA) otherwise, their incorporations were addressed.

Ethical approval

The current study does not require an ethical approval as the main materials are ovaries that collected from slaughtered animals in local abattoir according to Minister of Agriculture regulations.

Experimental design

The control groups K₁ (control for the good morphological quality group) and K₂ (control for the low morphological quality group) were subjected to an incubation temperature of 38.5°C. The treated groups of good quality oocytes K₃ and K₄ were exposed to 41°C and 42°C, respectively. The treated groups of low quality oocytes K₅ and K₆ were exposed to 41°C and 42°C, respectively. The four treated groups (about 260 oocyte/group) were exposed to the assigned temperature for the first 6 hours of *in vitro* maturation, thereafter, the incubation temperature for all treatments decreased to 38.5°C for 24 hours until the end of IVM duration. Maturation rates of the K₁, K₂, K₃, K₄, K₅, and K₆ oocytes were estimated based on the expansion of cumulus cells and the Pb extrusion ratio, then all groups were subjected to parthenogenetic activation to follow up early embryo developmental rate until blastocyst stage.

Procedures

Ovaries collection and oocytes recovery

Camel ovaries were collected from nearby abattoirs. The collected ovaries were transported to the laboratory within 3 hours in pre-warmed (30°C) physiological saline (0.9% NaCl) supplemented with (100 µg/ml streptomycin sulfate and 100 IU/ml penicillin). The collected ovaries were washed once in 70% ethanol and then three times in phosphate buffer saline supplemented with (100 µg/ml streptomycin sulfate and 100 IU/ml penicillin). Cumulus–oocyte complexes (COCs) were aspirated from follicles 2–8 mm in diameter using a 10 mL disposable syringe with an 18-gauge needle. The recovered COCs were washed three times in Hepes-buffered Medium199 (22340; Gibco, UK) supplemented with 100 µg/ml streptomycin sulfate, 100 IU/ml penicillin, and 10% inactivated fetal bovine serum (FBS). Cumulus–oocyte complexes were classified into good (grade A and B) and low quality (grade C) based on morphological assessment. The assessment of oocyte quality was based on the number of cumulus cell layers and the homogeneity of cytoplasm (Mesbah et al., 2016).

In vitro maturation of oocytes

The *in vitro* maturation medium is a basic maturation medium (TCM199) supplemented with 20 µg/mL follicle-stimulating hormone, 10% FBS, 10 ng/mL epidermal growth factor, 100 µg/ml streptomycin sulfate, and 100 IU/ml penicillin. The COCs were washed twice in IVM medium according to the experiment design. A total of 25–30 COCs/well were cultured in 400 µl of IVM medium overlaid with 400 µl mineral oil in 4 well culture plate (Nunc, Denmark) under 5% CO₂ and humidified air for 30 hours. Examination of matured oocytes was performed after 30 hours of incubation and based on cumulus expansion and extrusion of the first polar body using Optica stereomicroscope (SZM-LED1, Italy) and Leica inverted microscope.

Parthenogenetic activation and culture

The matured oocytes were denuded by gentle repetitive pipetting in a 0.1% hyaluronidase solution (Irvine Scientific, CA 92705 USA). Cumulus-free oocytes were then incubated in TCM-199 containing 10% FBS supplemented with 7% ethanol for 7 min (Wani, 2007) in a dark chamber. Oocytes were then washed in TCM-199 with 10% FBS, then transferred to 100-µL micro-drops of 4 mM 6-dimethylaminopurine (6-DMAP, D-2629), covered with mineral oil, and cultured in a humid atmosphere of 5% CO₂ at 38.5°C for 4 hours. After activation, activated oocytes were washed twice in the *in vitro* culture medium (Continuous Single Culture -NX Complete, cat no. 90168, Irvine Scientific, CA 92705 USA) placed into 4-well culture Plates (400 µl culture media per well) under mineral oil in the humidified atmosphere of 5% CO₂ at 38.5°C. Cleavage rate (2–8 cells) was recorded 48 hours after culture. The number of embryos developed to the blastocyst stage was assessed 7 days after *in vitro* culture.

Statistical analysis

Experimental Statistical analysis for maturation rate (expansion level and extrusion of the polar body) and embryonic development rates were performed using a randomized complete block design with six replications for each group using the web Agri Stat Package. The treatment means were compared by the least significant difference (LSD) test as reported by Snedecor and Cochran (1994) using ASSISTAT software, version 7.7 beta freeware. P-value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Effect of heat stress on maturation rate of camel oocytes

Climate change has raised tangible concerns about global warming representing a significant threat to the feasibility and sustainability of livestock farming worldwide, particularly in regions located in tropical and subtropical zones. Egypt is located in a subtropical area, where the intense heat stress of summer is accompanied by high ambient humidity. This issue worsens the situation and represents a constraint to farm animal welfare, productivity, and reproductive performance (Marai and Habeeb, 2010). In this regard, cumulus-oocyte complexes are highly affected when the female is exposed to environmental heat stress during follicular development, ovulation and *in vivo* events of oocyte maturation (Al-Katanani et al., 2002; Gendelman and Roth, 2012; Sadeesh et al., 2016).

There is a growing interest in establishing and improving assisted reproductive techniques, such as *in vitro* embryo production in camel. In addition, the *in vitro* maturation of oocyte is the key step for improving the success rate of *in vitro* embryo production in this species (Trasorras et al., 2013; Mesbah et al., 2016; Moulavi and Hosseini, 2018). Accordingly, cumulus expansion and extrusion of the first polar body are the main criteria used to evaluate the nuclear maturation of camel oocytes (Yaqoob et al., 2017; Saadeldin et al., 2019). The data of the current study indicated no change in the incidence of cumulus expansion in good quality COCs before ($92.98 \pm 0.95\%$) and after heat shock exposure at 41°C ($93.61 \pm 1.32\%$) and 42°C ($93.96 \pm 0.64\%$). However, the low quality COCs demonstrated no sign of cumulus expansion, which may be due to decreased number of cumulus layers in this quality category or low expression of TGF β genes. In support to this idea, the lowest first polar body extrusion rate ($11.2\% \pm 2.5\%$) was found in the group of camel oocytes treated with the inhibitor of the TGF β pathway (SB-431542) which interferes with the activity of activin receptor-like kinases accompanied by no expansion of cumulus cells (Saadeldin et al., 2019).

The results of this study indicated that the heat stress has the same effect on both good and low-quality oocytes concerning nuclear maturation represented by Pb extrusion percentage and cytoplasmic maturation represented by cumulus expansion score. The cumulus expansion level was significantly higher in good quality oocyte groups K1 ($P < 0.01$, $92.98 \pm 0.95\%$), K3 ($93.61 \pm 1.32\%$), and K4 ($93.96 \pm 0.64\%$) compared to low quality oocyte groups of K2 (0%), K5 (0%), and K6 (0%). Moreover, exposure of COCs to heat stress at 42°C significantly decreased the Pb ratio in K4 ($P < 0.01$, $20.73 \pm 0.49\%$) and K6 ($20.30 \pm 0.52\%$) compared to the control K1 ($32.31 \pm 0.54\%$), K2 ($30.98 \pm 1.36\%$), and heat-stressed groups at 41°C K3 ($31.44 \pm 0.92\%$) and K5 ($31.63 \pm 0.47\%$). Indeed, buffalo cumulus-oocyte complexes that were collected during hot season had a high percentage of arrested oocytes in metaphase I stage after *in vitro* maturation (Abdoon et al., 2014). Interestingly, bovine oocytes that have been exposed to heat shock at 40.0°C and 41.0°C recorded lower rates of nuclear and cytoplasmic maturation (Maya-Soriano et al., 2013). Similarly, El-Sayed et al. (2018) have indicated that exposure of buffalo COCs to heat shock reduced the percentage of *in vitro* matured oocytes at temperature of 39.5°C and 40.5°C compared with that of non-treated control group (38.5°C).

Effect of heat stress on the development rate of *in vitro* produced camel embryos from oocytes with different quality

After parthenogenesis, development rate until 2-8 cells embryo was lower ($P < 0.01$) for good (K3= 50 ± 0.92 and K4= 55 ± 1.58) and low (K5= 45 ± 1.28 and K6= 27 ± 0.56) quality oocytes exposed to heat stress, compared with control K1 (63 ± 1.28) and K2 (53 ± 1.85). Moreover, development rate up to 2-8 cells embryo was lower ($P < 0.01$) for low quality oocytes exposed to heat stress K5 (45 ± 1.28) and K6 (27 ± 0.56) compared with good quality oocytes exposed to the same heat stress K3 (50 ± 0.92) and K4 (55 ± 1.58). The development rate was significantly lower for control low quality ($P < 0.01$, 53 ± 1.85) compared to the control good quality oocytes (63 ± 1.28). Bovine oocytes exposed to heat stress at 40.0°C and 41.0°C reduced *in vitro* embryo development rate (Maya-Soriano et al., 2013). Saadeldin et al. (2018) demonstrated that camel oocytes exposed to short acute heat shock at 45°C for 2, showed reduction in maturation rate, linked with decreased ooplasmic diameter and increased percentage of chromosomal abnormalities.

The percentage of oocytes developed to the blastocyst stage was significantly lower for good quality oocytes exposed to heat stress K3 and K4 ($P < 0.01$), compared with control K1. Blastocyst rate was significantly lower for low quality oocytes exposed to heat stress 42°C K6 ($P < 0.01$), compared with low quality oocytes exposed to 41°C K5 and control low quality oocytes K2 ($0, 3 \pm 0.22$, and 9 ± 0.22 , respectively). Blastocyst rate was lower for control low quality oocytes K2 compared with control good quality oocytes K1 ($P < 0.01$, 9 ± 0.22 , and 15 ± 0.22 , respectively). There was no significant difference between good quality oocytes exposed to 42°C K4 and low-quality oocytes exposed to 41°C (2 ± 0.21 and 3 ± 0.22 , respectively). In support to previous observation, experimental exposure of COCs to heat shock during the maturation has revealed reduced cleavage rate as well as blastocyst development (Edwards and Hansen, 1997). In addition, *in vivo* experiments have reported reduced pregnancy rate by 25% for each °C elevation in body temperature, which is due to the negative impact of heat stress on preimplantation development embryos (Nabenishi et al., 2012). Recently, heat shock at 40°C for 24 h during IVM of bovine COCs had no effect on Pb extrusion rate however; it reduced the rate of embryo cleavage and blastocyst development (Pöhland et al., 2020).

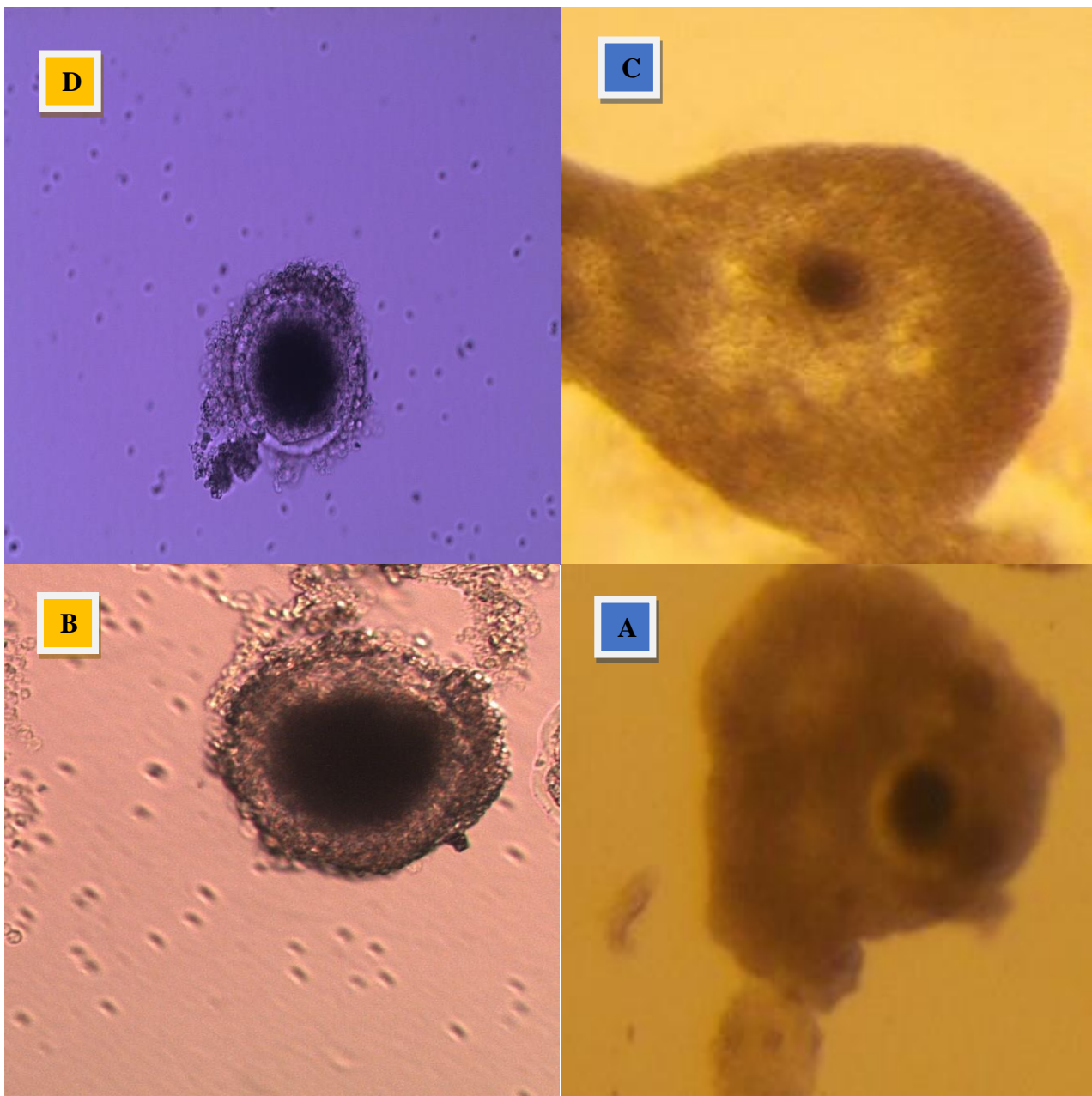


Figure 1. Immature and mature oocytes with different quality. **A:** Immature good quality oocyte. **B:** Immature low quality oocyte. **C:** Mature good quality oocyte. **D:** Mature low quality oocyte

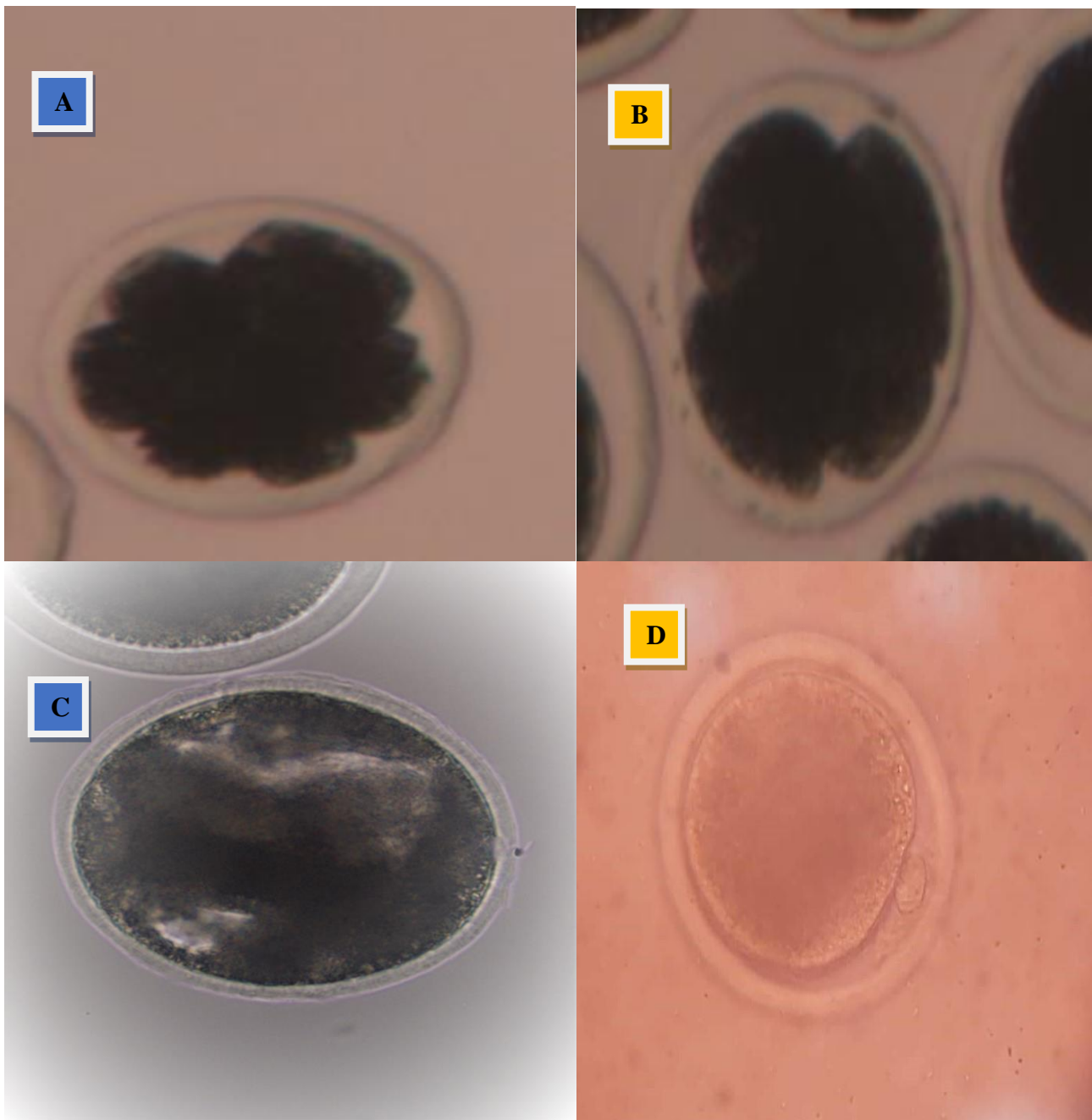


Figure 2. Polar body and different developmental stages of camel embryos. **A:** Blocked embryo. **B:** Four cell stage embryo. **C:** Blastocyst. **D:** Mature oocyte with polar body.

Table 1. Effect of heat stress on developmental rate of *in vitro* produced camel embryos from oocytes with different quality

group	Oocytes (n)	Cumulus expansion (%) [*]	Matured oocytes (n) ^{**}	Polar body extrusion (%) ^{***}	Polar body (n)
K1	266	92.98 ± 0.95 ^a	247 ± 6.34 ^a	32.31 ± 0.54 ^a	86 ± 2.25a
K2	273	0 ^b	0 ^b	30.98 ± 1.36 ^a	87 ± 2.77a
K3	230	93.61 ± 1.32 ^a	216 ± 4.03 ^a	31.44 ± 0.92 ^a	72 ± 1.21b
K4	275	93.96 ± 0.64 ^a	258 ± 7.01 ^a	20.73 ± 0.49 ^b	57 ± 1.59c
K5	282	0 ^b	0 ^b	31.63 ± 0.47 ^a	89 ± 2.47a
K6	222	0 ^b	0 ^b	20.30 ± 0.52 ^b	45 ± 0.85c

^{a,b}: Means within a column with different superscripts differ significantly (P < 0.01). ^{*}Evaluated after 30 h. ^{**}Based on cumulus cells expansion. ^{***}Based on polar body extrusion. K1: Good quality oocytes incubated at 38.5°C for 30 hours; K2: Low quality oocytes incubated at 38.5°C for 30 hours; K3: Good quality oocytes exposed to 41°C for the first 6 hours of maturation; K4: Good quality oocytes exposed to 42°C for the first 6 hours of maturation; K5: Low quality oocytes exposed to 41°C for the first 6 hours of maturation; K6: Low quality oocytes exposed to 42°C for the first 6 hours of maturation. n: Number

Table 2. Effect of heat stress on development rate of *in vitro* produced camel embryos from oocytes with different quality

group	Oocytes (n)	Development 2-8 cells (n)*	Blastocyst (n)**	blocked embryos (n) ***
K1	266	63 ± 1.28 ^a	15 ± 0.22 ^a	48 ± 1.06 ^c
K2	273	53 ± 1.85 ^c	9 ± 0.22 ^b	44 ± 1.36 ^d
K3	230	50 ± 0.92 ^b	6 ± 0 ^c	44 ± .92 ^c
K4	275	55 ± 1.58 ^{bc}	2 ± 0.21 ^{de}	53 ± 1.45 ^{ab}
K5	282	45 ± 1.28 ^d	3 ± 0.22 ^d	42 ± 1.06 ^b
K6	222	27 ± 0.56 ^e	0 ^e	27 ± 0.56 ^a

^{a,b,c,d,e} Means within a column with different superscripts differ significantly ($P < 0.01$). *Calculated as % of total oocytes. **Calculated as % of cleaved embryos. ***Calculated as % of cleaved embryos. K1: Good quality oocytes incubated at 38.5°C for 30 hours; K2: Low quality oocytes incubated at 38.5°C for 30 hours; K3: Good quality oocytes exposed to 41°C for the first 6 hours of maturation; K4: Good quality oocytes exposed to 42°C for the first 6 hours of maturation; K5: Low quality oocytes exposed to 41°C for the first 6 hours of maturation; K6: Low quality oocytes exposed to 42°C for the first 6 hours of maturation. n: Number

CONCLUSION

The results of this study indicated that the exposure of camel oocytes to heat stress during the first 6 hours of *in vitro* maturation could severely affect their developmental competence. The low morphologically quality COCs have no signs of cumulus expansion and had lower development competence than good quality after exposure to *in vitro* heat shock.

DECLARATIONS

Authors' contribution

All authors have contributed equally in laboratory works, the experimental design, writing, and revision of the manuscript.

Competing interests

All authors declare no competing interests that might interfere with the data provided in the current manuscript.

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