



The Impact of Follicular Fluid on the *in vitro* Maturation of Camel Oocytes

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ABSTRACT

Background: The objective of this research is to find out how much of an impact adding camel follicular fluid (CFF) to camel oocytes *in vitro* maturation (IVM) medium has on getting meiosis back on track.

Methods: Ovaries from camels were obtained at the central slaughterhouse in Riyadh, Kingdom of Saudi Arabia (KSA). Oocytes were extracted from follicles ranging in size from 2 to 8 mm. Camel oocytes were cultivated in a maturation medium containing varying amounts of camel follicular fluid: 0% (control), 2.5% and 5% for a duration of 32-36 hours.

Result: According to the result, the maturation rate of oocytes was considerably (p.05) lower in the 2.5% CFF and 5% CFF groups compared to the control group. It is advised that greater CFF concentrations of camel oocytes be added to the IVM culture in order to improve nuclear and cytoplasmic maturation and boost oocyte competency.

Key words: Camel, Cumulus cell, *In vitro* maturation, Oocyte, Ovaries.

INTRODUCTION

Many issues, maybe caused by insufficient final maturation, restrict embryo development *in vitro*. With respect to oocyte, a slow pace of embryonic development *in vitro*. So, it's safe to say that *in vitro* maturation has a significant impact on the success of embryo evolution. According to the study conducted by Wani and Nowshari in (2005), it was determined that eggs need to undergo a maturation process in the laboratory for a period of 32-44 hours in order to achieve the highest number of mature eggs in the MII stage, which ranges from 42-52%.

The *in vitro* maturation rate (IVM) is the only one of the three major processes that are involved in the *in vitro* embryo production (IVEP) technology. This rate is rather low when compared to the pace at which oocyte mature *in vivo*.

Lu *et al.* (2018), improving the microenvironment of the oocyte such that it is comparable to that of the *in vivo* counterparts is the most difficult obstacle that IVEP must overcome. On the other hand, certain IVM components and conditions can be necessary (Watson *et al.*, 2000; Barakat *et al.*, 2015; Barakat *et al.*, 2016; Al-Mutary *et al.*, 2019). have all found that there are certain variations in the gene expression of oocytes that result in a decline in the quality of the oocytes.

Various chemicals have been added to enhance the culture conditions of the *in vitro* maturation (IVM) process. One of these substances is the follicular fluid (FF), which contains a variety of nutrients that might potentially impact the development of the oocyte growth, fertilization and embryo development (Germoush *et al.*, 2018).

FF has been utilized in intravenous methanol (IVM) studies in a variety of animals and plants, including cattle (Ali *et al.*, 2004), goats (Masudul *et al.*, 2012), pigs (Ito *et al.*, 2008), buffalo (Gupta *et al.*, 2005), horses

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(Bogh *et al.*, 2002) and sheep (Sun *et al.*, 1994). Nonetheless, oocyte and blastocyst gene expression may be affected by adding FF to *in vitro* maturation medium, which in turn affects the amount of messenger RNA (Watson *et al.*, 2000; Cruz *et al.*, 2014).

However, the influence of camel follicular fluid (CFF) supplementation during the *in vitro* maturation of camel oocytes on maturation has not been examined. In general, the competence of an oocyte is measured by the maturation rate, the fertilization rate, the cleavage rate and the blastocyst ratio (Ammari *et al.*, 2022a). Within the scope of this study, we investigated the impact that CFF has on oocyte maturation.

MATERIALS AND METHODS

An aspiration was used to obtain the follicular fluid from intact follicles that were discovered in camel ovaries that had been taken from animals that had been slaughtered at a slaughterhouse more recently. The fluid was collected in 15 ml centrifuge tubes and subjected to two rounds of centrifugation at 4000 rpm for 20 minutes each to eliminate cellular debris. The liquid portion was collected, passed

through a 0.45 mm filter and subsequently rendered inactive by heating at 56°C for 30 minutes. It was then kept in separate portions in 1.5 ml Eppendorf tubes at a temperature of -20°C until needed (Al-Mutary *et al.*, 2019).

Following oocyte collected from the ovaries, (COCs) were separated into two groups, with the distinction between the two groups being determined by the amount of camel follicular fluid (CFF) that was added to IVM medium: The control group had 0% CFF, 2.5% CFF and 5% CFF. Repeated pipetting was used to remove the cumulus cells that were surrounding the oocytes after they had matured. The oocytes were then placed in Hank's medium that had been supplemented with 100 IU/ml of hyaluronidase.

A local slaughterhouse located south of the city of Riyadh was be used to collect ovaries from adult females who have recently been slaughtered. These ovaries were then be brought to the laboratory in a container that contains 0.9% normal saline and was be kept at room temperature normal according to Ammari *et al.* (2022b).

The ovaries washed twice with a salt solution once they get to the lab. Then, the method called the Aspiration Method used to remove the oocyte from the main follicles, which were 2 to 8 mm in diameter. 10 ml syringe was connected to 19 diameter needles. Just before extraction, the oocyte collection solution, Hank's Salt's TCM-199, which is about half a milliliter in volume, was added to the syringe. The contents of the syringe will be carefully placed into a 15 ml conical tube after the drawing operation. After that, the oocyte was allowed to settle to the bottom of the tube by leaving it in the solution for 5 minutes. We remove the fluids. Then, use a clean 90 mm petri dish to transfer the remaining material containing the COCs. Next, gently we transferred the COCs (multi-layered with homogeneous cytoplasm) to the Petri plate containing the oocyte collection medium using a glass pipette. Utilizing TCM-199 in conjunction with glutamine, 25 mM of 4-(2-hydroxyethyl) and Hank's salts forms the handling media. A -1 piperazineethanesulfonic acid (HEPES) supplement, 10% fetal calf serum, 0.3 mM sodium pyruvate, 25 mg/mL gentamicin sulfate and 143 mg/mL heparin sodium salt are all components of the prepared solution according to Ammari *et al.* (2023).

The oocyte samples from each group, which had been stripped of their outer layer, were placed on glass slides. The slides were then coated and secured with a mixture of acetic acid and ethanol in a ratio of three parts acetic acid to one-part ethanol (v/v). This fixation process lasted for twenty-four hours and then stained with 1% aceto-orcein stain. Examples of the samples were taken from each group. An examination of the oocytes was carried out with the assistance of a phase contrast microscope (400x Olympus, CKX41 Japan). The nuclear state of the oocytes was evaluated, which included the presence of germinal vesicles, germinal vesicle breakdown, metaphase I and metaphase II (Fig 1).

The statistical analysis of the data involved using the SPSS version 20 software program. A minimum of three duplicate trials were conducted for each treatment comparison. The analysis was performed by SPSS Inc., based in Chicago, IL, USA. To begin the data analysis process, we utilized the Kolmogorov-Smirnov test to normalize the data. The means of GV, GVBD, MI, MII and degenerated oocytes throughout *in vitro* maturation were compared across all groups using one-way analysis of variance (ANOVA) and post-LSD Dunnett's test.

RESULTS AND DISCUSSION

According to the findings, there was a highly significant rise in the level of cumulus cell proliferation in 5% CFF and 2.5% CFF when compared to the control, however the difference was not statistically significant (Fig 1).

The results reveal that the maturation rate is unaffected by the two groups' varying follicular fluid concentrations, although it is considerably ($P \leq 0.5$) slowed down in the 2.5% and 5% CFF groups. At the same time, compared to the control groups, the 2.5% CFF and 5% CFF groups had larger proportions of GV, GVBD and MI oocytes (Fig 2).

Follicular fluid includes several essential hormones and growth factors that aid in the development of oocytes *in vivo*. The addition of follicular fluid to camel oocyte *in vitro* maturation media may boost oocyte competence. Numerous studies have demonstrated that the incorporation of follicular fluid into the maturation medium results in an increase in the maturation rates of oocytes in a variety of mammalian oocytes (Coleman *et al.*, 2007; Sun *et al.*, 1994; Caillaud *et al.*, 2008; Al-Mutary *et al.*, 2019).

Our results agree with the results of researchers (Yaqoob *et al.*, 2017) who used follicular fluid at a concentration of 10% in the medium of the ripening environment and there was an increase in the expansion of cumulus cells, but not significantly effect on blastocyst % after PA.

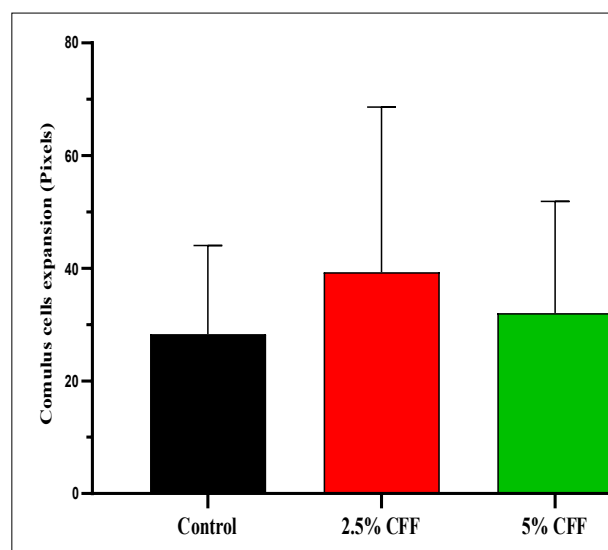


Fig 1: Percentage of cumulus cells expansion.

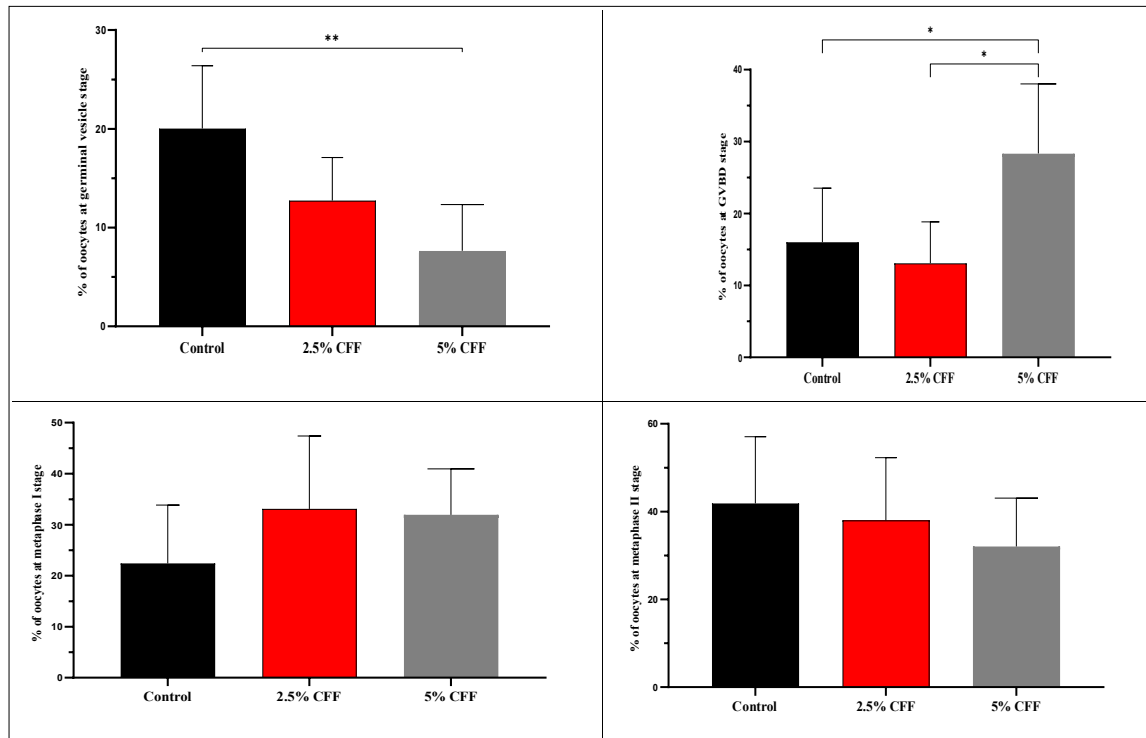


Fig 2: The impact of supplementing the *in vitro* maturation medium with CFF on the maturation rates of camel oocytes.

In spite of the fact that supplementation with 10% FF led to an improvement in cumulus expansion, there was no discernible impact on the developmental competence of the activated oocytes involved. Cumulus growth, on the other hand, is advantageous because it makes it simpler to remove cumulus cells from oocytes in preparation for further oocyte handling. This, in turn, mitigates the adverse effects of extended exposure to hyaluronidase (Ishizuka *et al.*, 2014).

CONCLUSION

The results of our study indicate that adding follicular fluid to the maturation medium did not cause clear significant differences. This may be the reason for the decrease in the concentrations used, but on the contrary, the cumulus cells showed a clear expansion compare with the control group, which gives an indication that there is maturation at the cytoplasmic level, which encourages further studies and the use of higher concentrations may improve the maturation rates of camel oocyte.

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Conflict of interest

All authors declared that there is no conflict of interest.

REFERENCES

- Ali, A., Coenen, K., Bousquet, D. and Sirard, M.A. (2004). Origin of bovine follicular fluid and its effect during *in vitro* maturation on the developmental competence of bovine oocytes. *Theriogenology*. 62: 1596-1606.
- Ammari, A.A., Alhimaidi, A.R., Amran, R.A., Al-Ghadi, M.G. and Rady, A.G. (2023). Seasonal influence on oocyte recovery rate, quality and *in vitro* maturation in cows. *Indian Journal of Animal Research*. 57(9): 1139-1142. doi: 10.18805/IJAR.BF-1632.
- Ammari, A.A., Amran, R.A., Al Ghadi, M.G. and Alhimaidi, A.R. (2022a). Morphometric assessment of the bovine ovary for *in vitro* matured oocyte quality to determine developmental competence. *Indian Journal of Animal Research*. 56(5): 557-562. doi: 10.18805/IJAR.BF-1471.
- Ammari, A.A., ALGhadi, M.G., Amran, R.A., Al Malahi, N.M. and Alhimaidi, A.R. (2022b). Interspecific Nuclear Transfer Blastocysts Reconstructed from Arabian Oryx Somatic Cells and Domestic Cow Ooplasm. *Veterinary Sciences*. 10(1): 17. doi: 10.3390/vetsci10010017.
- Al-Mutary, M., Al-Ghadi, M., Al-himaidi, A., Iwamoto, D., Al-Anazi, Y., Ammari, A., Ahmad, J., Al-Khedhairi, A. (2019). Using RT-PCR and glutathione level to study the effect of follicular fluid on *in vitro* maturation and gene expression of sheep oocytes. *Saudi J. Biol. Sci.* 26(6): 1216-1222.
- Barakat, I.A.H., Khalil, W.K.B. and Al-Himaidi, A.R. (2015). *Moringa oleifera* extract modulates the expression of fertility related genes and elevation of calcium ions in sheep oocytes. *Small Rumin.* 67-75.

- Barakat, I.A.H., Khalil, W.K.B. and Al-Himaidi, A.R. (2016). Curcacycline A and B modulate apoptosis induced by heat stress in sheep oocytes during *in vitro* maturation. Small Rumi. 187-196.
- Bogh, I.B., Bezard, J., Duchamp, G., Baltzen, M., Gerard, N., Daels, P. and Greve, T. (2002). Pure preovulatory follicular fluid promotes *in vitro* maturation of *in vivo* aspirated equine oocytes. Theriogenology. 57: 1765-1779.
- Caillaud, M., Dell'Aquila, M. E., De Santis, T., Nicassio, M., Lacalandra, G.M., Goudet, G. and Gerard, N. (2008). *In vitro* equine oocyte maturation in pure follicular fluid plus interleukin-1 and fertilization following ICSI. Animal Reproduction Science. 106(3-4): 431-439.
- Coleman, N.V., Shagiakhmetova, G.A., Lebedeva, I.Y., Kuzmina, T.I. and Golubev, A.K. (2007). *In vitro* maturation and early developmental capacity of bovine oocytes cultured in pure follicular fluid and supplementation with follicular wall. Theriogenology. 67(5): 1053-1059.
- Cruz, M.H.C., Saraiva, N.Z., Cruz, J.F.D., Oliveira, C.S., Collado, M.D., Fernandes, H., Castro, F.C.D. and Garcia, J.M. (2014). Effect of follicular fluid supplementation during *in vitro* maturation on total cell number in bovine blastocysts produced *in vitro*. Rev. Brasil. Zootec. 43: 120-126.
- Ishizuka, Y., Takeo, T., Nakao, S., Yoshimoto, H., Hirose, Y., Sakai, Y., *et al.* (2014). Prolonged exposure to hyaluronidase decreases the fertilization and development rates of fresh and cryopreserved mouse oocytes. Journal of Reproduction and Development. 60(6): 454-459.
- Germoush, M.O., Al-Mutary, M.G., Al-Himaidi, A.R., Al-Ghadi, M. G., Iwamoto, D., Al-Anazi, Y., *et al.* (2018). Effects of follicular fluid on developmental competence and gene expression of *in vitro* fertilized sheep embryos. Pakistan Journal of Zoology. 50(4): 1267-1277.
- Gupta, P.S.P., Ravindra, J.P., Kumar, V.G., Raghu, H.M. and Nandi, S. (2005). Stimulation of *in vitro* ovine oocyte maturation with a novel peptide isolated from follicular fluid of the buffalo (*Bubalus bubalis*). Small Rumin. Research. 59: 33-40.
- Ito, M., Iwata, H., Kitagawa, M., Kon, Y., Kuwayama, T. and Monji, Y. (2008). Effect of follicular fluid collected from various diameter follicles on the progression of nuclear maturation and developmental competence of pig oocytes. Anim. Reprod. Sci. 106: 421-430.
- Lu, C., Zhang, Y., Zheng, X., Song, X., Yang, R., Yan, J., Feng, H., and Qiao, J. (2018). Current perspectives on *in vitro* maturation and its effects on oocyte genetic and epigenetic profiles. Sci. China Life Sci. 61(6): 633-643.
- Masudul, H., Khandoker, M.A.M.Y., Kabiraj, S.K., Asad, L.Y., Fakruzzaman, M. and Tareq, K.M.A. (2012). Effect of goat follicular fluid on *in vitro* production of embryos in black Bengal goats. Iranian J. Appl. Anim. Sci. 2: 287-294.
- Sun, F.J., Holm, P., Irvine, B. and Seamark, R.F. (1994). Effect of sheep and human follicular fluid on the maturation of sheep oocytes *in vitro*. Theriogenology. 41: 981-988.
- Wani, N.A. and Nowshari, M.A. (2005). Kinetics of nuclear maturation and effect of holding ovaries at room temperature on *in vitro* maturation of camel (*Camelus dromedarius*) oocytes. Theriogenology. 64(1): 75-85.
- Watson, A.J., De Sousa, P., Caveney, A., Barcroft, L.C., Natale, D., Urquhart, J. and Westhusin, M.E. (2000). Impact of bovine oocyte maturation media on oocyte transcript levels, blastocyst development, cell number and apoptosis. Biol. Reprod. 355-364.
- Yaqoob, S.H., Saadeldin, I.M., Swelum, A.A.A., and Alowaimier, A. N. (2017). Optimizing camel (*Camelus dromedarius*) oocytes *in vitro* maturation and early embryo culture after parthenogenetic activation. Small Ruminant Research. 153: 81-86.