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Cairo University Faculty of Veterinary Medicine Department of Theriogenology

Improving the developmental potential of vitrified/warmed dromedary camel oocytes

Thesis presented By

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For the degree of M. V. Sc. (Theriogenology)

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Super-vision sheet

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Abstract

Vitrification of dromedary camel oocytes provides a potential resource for female gametes in face of seasonality of reproduction and low fertility. However, research on assisted reproductive techniques is still in its early stages in camels when compared to other farm animals. Cryoprotectant toxicity, osmotic imbalance, and oxidative stress are the main challenges during vitrification-warming procedures notably in oocytes due to high lipid and water contents. The experimental design herein was addressed to overcome the major challenges associated with the vitrification process to enhance the outcomes of this method post warming through: (I) reducing the CPAs quantity (II) osmolytes supplementation or (III) antioxidant supplementation, which consequently could improve the viability, maturation, cleavage, and blastocyst rates of vitrified/ warmed immature dromedary camel oocytes. For this purpose, 3366 dromedary camel cumulus-oocyte complexes (COCs) were aspirated from 1260 ovaries, obtained from a local abattoir (Cairo, Egypt) from February 2021 to May 2022. The COCs, with at least one to three layers of compact cumulus cells and a homogenous ooplasm were selected under a stereomicroscope and randomly allocated into groups according to each experiment applied as follows: The first experiment: The results showed that omitting trehalose from the vitrification solution (VS) could positively impact on the developmental potential of vitrified/warmed immature dromedary camel oocytes. The percentage of viable oocytes was significantly higher (P < 0.05) in VS without trehalose than in VS containing 0.5 mM trehalose. Nuclear maturation, cleavage (48 h postinsemination; pi), and blastocyst rates (7 days pi) were significantly higher (P < 0.05) in the trehalose-free group. No significant differences were observed in oocyte maturation and development rates between the trehalose-free group and control groups. The second experiment: The data revealed that supplementation of 1 mM of glycine to the vitrification solution of vitrified/warmed immature dromedary camel oocytes produced the best (P< 0.05) outcomes compared to the other concentrations tested (0.5- and 2-mM glycine) in the following parameters: viability (90%), nuclear maturation (75.7%), cleavage 48h- post-insemination (35.8%), blastocysts/oocyte (18.5%), and blastocysts/cleaved embryos rates (51.7%). The third experiment: Firstly, different concentrations of CoQ10 (25, 50, and 100 µM) were added to IVM media. The findings indicated that the concentration of 50 µM of CoQ10 significantly advanced cumulus cells' full expansion, MII, cleavage, and blastocyst rates. Based on these results, 50 µM of CoQ10 was used in the next experiment and added to VS and/or IVM, where; Vit ± (i.e., V.S ± CoQ10), IVM ± (i.e., IVM ± CoQ10). COCs were assigned randomly to one of four groups: a) Vit - / IVM - group (control); b) Vit + / IVM - group; c) Vit - / IVM + group; d) Vit + / IVM + group. The findings from such experiment revealed that Vit - / IVM + group showed improvement (P< 0.05) in the post-warming viability and preimplantation potential in vitro that were augmented by the highest (P< 0.05) TAC and the lowest (P< 0.05) MDA levels in the remaining media.

Keywords: Camel, CoQ10, Glycine, Trehalose, Oocytes, Vitrification.

Dedication

To My Father,

My Mother,

My Brothers,

My Lovely Sister,

and My Future Wife.

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