

شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلو

بسم الله الرحمن الرحيم





MONA MAGHRABY



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EFFECT OF OXIDATIVE STRESS ON DEVELOPMENT OF IN VITRO PRODUCED CAMEL EMBRYOS

By

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Camel Embryos.

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ABSTRACT

The aim of the present study was to evaluate the effect of supplementation of ascorbic acid (first experiment), cysteine (second experiment) and combination of both antioxidants (third experiment) to in vitro culture (IVC) medium on the development of dromedary camel embryos and expression profile of candidate genes regulating embryo quality. Cumulus-oocyte complexes (COCs) were recovered from camel ovaries collected from local abattoir. Good quality COCs were used in the standard protocol of in vitro embryo production including in vitro maturation (IVM) and in vitro fertilization (IVF). The presumptive zygotes were incubated in culture medium with/without antioxidant according to the experiment. First experiment: The presumptive zygotes were allocated into four treatments of ascorbic acid, namely; control group (T1), 50 µg/ml (T2), 100 µg/ml (T3) and 150 μg/ml of ascorbic acid (T4). In the second experiment: three cysteine concentrations (T2: 100 µM, T3: 500 µM and T4: 1 mM) were supplemented to IVC medium and compared with control group (T1). The third experiment consisted of four experimental groups; control group without antioxidant (T1), 150 µg/ml ascorbic acid (T2). 100 μM cysteine (T3) and a combination of 150 μg/ml ascorbic acid and 100 μM cysteine (T4). The relative abundance of b-cell lymphoma 2 (BCL2), catalase (CAT), superoxide dismutase (SOD), glucose transporter 1 (GLUT-1), thioredoxin (TXN) and tumor suppressor protein (P53) genes were assessed using real-time PCR. All experimental groups were incubated in 5% CO₂, 20% O₂ and moist atmosphere at 38.5 °C for 6 days. In the first experiment, results showed that all groups supplemented with ascorbic acid (T2, T3 and T4) were significantly higher in blastocyst rate compared to the control group (10%, 16.67%, 25% and 2.04%, respectively). Meanwhile, no significant difference was observed among ascorbic acid treatments. In the second experiment: supplementing IVC medium of dromedary camel embryos with cysteine was found to have a positive significant effect on cleavage and blastocyst rates compared with control. Blastocyst rates were T1: 3.03%, T2: 22.22%, T3: 8.33% and T4: 6.25%. In the third experiment: blastocyst rates were T1: 2.04%, T2: 24%, T3: 22% and T4: 17.78%. Gene expression data supported the positive effects of antioxidant supplementation to IVC media on enhancing embryo development. In conclusion, supplementation of ascorbic acid (150 µg/ml) and/or cysteine (100 µM) to the IVC media had a beneficial effect on IVP camel embryos which could be due to a protection mechanism against apoptosis and reactive oxygen species (ROS) accumulation according to the analysis of apoptosis and oxidative stress related genes.

Key words: in vitro, embryos, dromedary, culture medium, antioxidants

DEDICATION

I dedicate this work to my mother, my father, and my wife for all the support and sacrifices they happily offered for my sake in order to reach this moment.

Also, I dedicate this work to my gifts from "ALLAH"; my beautiful daughter and beloved son.

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