DOSE SPECIFIC EFFECTS OF IONOMYCIN ON PARTHENOGENETIC ACTIVATION OF in vitro MATURED DROMEDARY OOCYTES

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ABSTRACT

Evaluation of the dose-specific effects of ionomycin on the parthenogenetic activation of Camelus dromedarius oocytes was done in the present study. Ovaries were collected from a local slaughterhouse. Cumulus-oocyte-complexes (COCs) were aspirated and cultured in a commercial IVM medium (BO-IVM) for 42 hrs in a humidified atmosphere containing 5% CO2 at 38°C. All metaphase II oocytes were activated either 2.5 µM, 5.0 µM or 10.0 µM of ionomycin for 4 min. Activated oocytes were immediately incubated in 2.0 mM 6-dimethylaminopurine (6-DMAP) in a commercial embryo culture medium (BO-IVC) for 4 hrs at 39°C in a humidified incubator with 5% CO2. Oocytes were cultured in BO-IVC for 7 days and developmental stages were monitored and recorded. After 42 hrs of in vitro maturation, 71.28% of oocytes were found with the extruded first polar body (metaphase II oocytes). The oocytes in the 5.0 µM of ionomycin group showed the highest blastocyst formation rate (56.99%) compared to the 2.5 µM (6.58%) and 10.0 µM (1.81%) groups. We recommended 5.0 µM of ionomycin for 4 min followed by incubation in 2.0 mM 6-DMAP for 4 hrs to activate camel oocytes.

Key words: Camel, in vitro maturation, ionomycin, oocytes, parthenogenetic

Parthenogenesis provides valuable insights into genomic imprinting during early embryonic development and it is helpful to understand the effectiveness for activation of oocytes. Among those, general principles of cell signaling systems (Solter, 1998; Ma et al, 2005). Furthermore, artificial activation of oocytes has a functional role in somatic cell nuclear transfer process as reconstructed oocyte needs to be activated for further embryonic development (Campbell, 1999; Kishikawa et al, 1999).

Following fertilisation, the intracellular calcium oscillation leads to cortical reaction and resumption of meiosis and formation of the second polar body to the effectiveness for activation of oocytes. Among those, perivitelline space (Küpker et al, 1998). Parthenogenesis mimics the functions of spermatozoa during fertilisation (Nakada and Mizuno, 1998). Different methods, such as chemical, mechanical or physical have been used for the creation of parthenogenetic embryos (Machaty, 2006; Brevini and Gandolfi, 2008). Some chemicals such as strontium (Cuthbertson et al, 1981) and ionomycin release calcium from cytoplasmic stores to increase intracellular free calcium, while electrical stimulus causes the influx of calcium from the extracellular medium and some chemicals such as ethanol promote both effects (Loi et al, 1998).

Various chemical agents have been tested for the resumption of meiotic II arrest and proved effective for activation of oocytes. Among those, calcium ionophores, such as ionomycin and A23187, together with protein synthesis or kinase inhibitors, such as 6-dimethylaminopurine (6-DMAP), is the most popular method for oocytes parthenogenesis in several different species (Heindryckx et al, 2009). Although, ionomycin has been previously used to derive parthenogenetic camel embryos, even SCNT-reconstructed oocytes were also activated with ionomycin (Wani, 2008; Hossein et al, 2021; Son et al, 2022), the optimum treatment conditions of ionomycin have not been studied systematically in camels. Accordingly, the objective of the present study was to evaluate the dose-specific effects of ionomycin treatment on the parthenogenetic activation of dromedary oocytes.

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