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Original article

# Calcium ($Ca^{2+}$) Expression and Intensity on Cumulus-Oocyte Complex (COCs)Kacang Goat After vitrification

Widjiati Widjiati[1*], Zakiyatul Faizah[2], Ninik Darsini[2], ViskiFitri Hendrawan[3], HellyNurul Karima[4], Choirunil Chotimah[4], Sutiman B. Sumitro[5],LitaRakhma Yustinasari[6], A. A. Muhammad Nur Kasman[7], JulianoMwenda Ntoruru[8], Epy Muhammad Luqman[6]

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## Abstract

The process of vitrification of the cumulus-oocyte complex (COCs) often results in cold shock.. When warming, heat shock occurs which can disrupt the balance of intracellular calcium ($Ca^{2+}$) intensity. Drastic changes in temperature cause Reactive Oxygen Species (ROS), So that it will affect changes on $Ca^{2+}$ in COCs. The role of calcium is needed for oocyte activation in the fertilization process. The purpose of this study was to measure the expression of $Ca^{2+}$ and the intensity of $Ca^{2+}$ in COCsafter vitrification. The study was divided into 2 groups, namely the control group (C) of fresh COCs, and the treatment group(T) of COCsafter vitrification. After vitrification for 24 hours, then thawing, then examined the expression of $Ca^{2+}$ by Immunocytochemistry method and the intensity of calcium ($Ca^{2+}$) with Confocal Laser Scanning Microscope (CLSM). The research data obtained were analyzed statistically by T-Test. The results showed that the expression of $Ca^{2+}$ in the control group (12.00±0.00) was different from the treatment group (0.35±0.79). The intensity of $Ca^{2+}$in the control group (1059.43±489.59) was different from the treatment group (568.21± 84.31). In conclusion of this study is that cryopreservation affects calcium in COCs, there were differences in the expression and the intensity of $Ca^{2+}$ between fresh COCsand COCs after vitrification. $Ca^{2+}$ intensity of COCsafter vitrification was concentrated in the nucleus, while fresh COCsin the cytoplasm.

**Keywords:** Calcium ($Ca^{2+}$), Cumulus-oocyte complex (COCs), food production, Kacang goat, Confocal Laser Scanning Microscope.

## Introduction

Kacang goat is one of Indonesia's germ-plasm, where its body shape is small and economically may not be profitable compared to foreign goats, but it has several advantages, including being easy to adapt because it is native to Indonesia and maintenance costs are not expensive. The breakthrough technology to produce goat embryos quickly can be done in vitro using the In Vitro Fertilization (IVF) method either conventionally by adding mature eggs with spermatozoa outside the body or using the Intra-Cytoplasmic Sperm Injection (ICSI) method. The main obstacles to goat embryo production through conventional IVF are low oocyte quality and limited oocyte sources for in vitro goat embryo production (Nasar et al. 2007).

Oocytes as a source of female gamete cells can be stored before maturation as an oocyte bank. Oocyte bank is a breakthrough for providing gamete cells for in vitro fertilization. Storage of oocytes for a long time for oocyte bank purposes can be done by cryopreservation. The best oocyte cryopreservation method can be done by the vitrification method (Rienzi et al., 2017). The vitrification method is often used because there is no crystal ice formation and the processing time is faster than the slow freezing method (MunckandVajta. 2017). According to Tavukcuoglu et al. (2012), the drawback of the vitrification method is that osmotic stress and toxic content in cryoprotectants can also reduce the level of oocyte quality.

Chen and Yang (2009) explained that both the slow-freezing method with increased sucrose concentration and new vitrification techniques significantly improve the results of cryopreservation of human oocytes. The survival of cryopreserved oocytes ranged from 74% to 90% using the slow-freezing method and from 84% to 99% by vitrification method. Overall, the survival rate of oocytes from vitrification (95%, 899/948) appeared higher than that of the slow-freezing method (75%, 1,275/1,683).For both protocols, firstthe cells must be treated with a combination of cell-permeating and non-permeating agents to minimizeice formation that can severely damage the cell. The stark difference between the success of oocyte and embryo

Widjiati_Main Manuscript_Revised - Word

**Original article**

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During the vitrification process, cryoprotectants are needed to protect oocytes both extracellularly and intracellularly from drastic temperature changes. This temperature change will cause an increase in Reactive Oxygen Species (ROS). Likewise, when warming up, a heat shock will occur. The heat shock occurs when there is a change in temperature from cold to warm. Increased ROS during thawing will cause damage to mitochondrial function and changes in $Ca^{2+}$ ion influx (Favetta et al. 2017).

Changes in temperature due to vitrification and during thawing will highly affect the intensity of $Ca^{2+}$ ions. The role of $Ca^{2+}$ ions in oocytes is very important related to the oocyte maturation process. In the maturation process, there will be the maturation of the nucleus and cytoplasm. In the nucleus maturation process, there will be an increase in $Ca^{2+}$ influx into the oocyte nucleus,so that protein regulators will run for the nuclear maturation process to occur. Calcium ($Ca^{2+}$) is necessary for the fertilization process and embryo development. The purpose of this study was to measure the expression of $Ca^{2+}$ and the intensity of $Ca^{2+}$in cumulus-oocyte complex.

The immunocytochemistry (ICC) method can observe the increase in $Ca^{2+}$ expression. The extracellular $Ca^{2+}$ expression can only be observed using the ICC method, while the intracellular $Ca^{2+}$ intensity uses the Confocal Laser Scanning Microscope method. The combination of the immunocytochemical method and the Confocal Laser Scanning Microscope can accurately measure the content and position of $Ca^{2+}$ in oocytes, due to the freezing process using the vitrification method.

## Materials and Methods

### Ethical Eligibility

The ethical Eligibility of this research was obtained from the Faculty of Veterinary Medicine, Universitas Airlangga by ethics number 1.KE.061.04.2019.

### Sample

The sample of this study was the ovary obtained from the waste of a slaughterhouse in Surabaya. Ovarian samples after being cleaned of tissue and blood were then washed with PBS. The clean ovary was placed in a bottle containing PBS solution and brought to the laboratory

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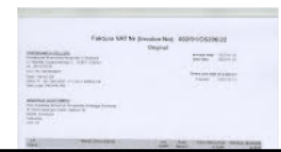

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