Study of Oviduct Expression Specificct Glycoprotein1 (OVGP1) on Oocyte and Goat Follicles (*Capra hircus*)

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Abstract: Oviduct Specific Glycoprotein (OVGP1) is a glycoprotein that has been identified as a protein secreted from unciliated secretory epithelial cells in oviducts with a molecular weight of 65 kDa in goats. The expression of this protein is very dependent on estrogen levels and the oestrus phase of the species. On the other hand, glycoprotein plays an important role in the process of oocyte maturation, spermatozoa capitation, and early embryonic development. The purpose of this research was to find the location of OVGP1 expression in oocytes and follicles of kacang goat (Capra hircus), an endogenous goat from Indonesian . The methods used in this study are: immunocytochemical techniques to see OVGP1 expression on oocytes and on goat follicles. OVGP1 expression in goat oocytes (Capra hircus) observed using immunocytochemical techniques was detected in the cumulus ooporus section, the zona pellucida, perivitelline space, and plasma oocyte membrane, whereas in the follicle the OVGP1 expression observed using immunohistochemical methods showed that OVGP1 was expressed in granulosa cells, external and internal theca cells.

1 INTRODUCTION

Polyspermia is the process of fusion of eggs by two or more consecutive spermatozoa (polyspermy) is a lethal condition in most organisms (Frank, 2000). Polyspermi, the most common abnormality found in fertilization, usually results in embryonic death. Physiologically, the penetration of the cytoplasm of an egg by more than one spermatozoa occurs in various species including insects, reptiles, and birds, whereas in mammals polyspermy is considered an abnormal phenomenon that results in zygote failure (Pepi et al., 2010). Mammals have several mechanisms to reduce the incidence of polyspermy. It is said that capacitation, spermatozoa transport through several parts of the female channel (ie: cervix, uterotubal junction), and reservoir of spermatozoa in the oviduct regulates the number of spermatozoa that reach the site of fertilization in various species. In pigs, in particular, gamete exposure to oviduct epithelial cells and oviduct secretion can reduce the occurrence of polyspermy (Gardner and Evans (2006). In addition to involving the female reproductive tract, the egg itself blocks the polyspermy to prevent fertilization by other spermatozoa after the egg has first fertilized the egg. Polyspermi inhibition in the egg occurs at two levels, namely: 1) at the plasma level of the egg cell membrane (oolemma) or vitellin and 2) on the outer layer of the egg called the zona pellucida (ZP) in mammals and vitelline envelope in non-mammals. At the level of the zona pellucida, this defense is often called a blockade zone (zone reaction) which involves cortical exocytosis of granules after oocyte penetration by spermatozoa, while at the oolemma level it is called a vitellin block which involves Ca^{2+} signaling (Coy and Aviles, 2010). After the penetration of spermatozoa, cortical granules (CG) release their contents into the perivitelline space (perivitelline space) in an event called a cortical reaction. The removal of CG contents changes the property of the zona pellucida known as the zone reaction, thereby blocking polyspermy penetration (Pepi *et al*., 2010).

Oviduct Specific Glycoprotein (OVGP1) is a glycoprotein that has been identified as a protein secreted from unciliated secretory epithelial cells in oviducts with a molecular weight of 65 kDa in goats

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(Pratiwi, 2017). Expression of this protein is very dependent on estrogen levels and the oestrus phase of the species, therefore this protein is often referred to as estrogen-dependent glycoproteins (OGPs). McCauley *et al*., (2003) also mentioned that OVGP1 expression in rats and pigs was localized in the zona pellucida, perivitelline space, and oocyte membrane plasma taken from the oviduct (in vivo) in the pre embryonic period.

In the study of Coy *et al*., (2008) states that OVGP1 added to cattle and pig oocytes during oocyte maturation in vitro is known to increase blockade of the zona pellucida and can reduce the incidence of polyspermy so that it can increase the success of fertilization. Seeing the potential role of OVGP1 which is very supportive in fertilization and seeing that research on OVGP1 in goats is still rarely done, it is necessary to conduct research on OVGP1 expression in the ovaries and OVGP1 expression in oocytes, in order to obtain supporting data on increasing fertilization rates in the process of in vitro fertilization.

2 MATERIALS AND METHODS

2.1 Tools and Materials

The research material in the form of ovaries was taken from female goat reproductive organs waste obtained from RPH in Malang, while oocytes were obtained from aspirations from reproductive organs waste cut in the RPH.

2.2 OVGP1 expression on Oocytes

The immunocytochemical technique used is the avidin-biotin-peroxidase-complex (ABC) method. Oocytes that are included in the quality of the Fixation on top of polylysine glass objects that have been given paraffin and vaseline at all four ends, then covered with a glass cover while pressing slowly. Oocytes are fixed in a solution of ethanol: acetic acid with a composition of 3: 1, for 2-3 days. After fixation, oocyte preparations are taken and then placed on a tissue to dry. Oocytes are observed under a microscope to see oocytes. After that, the aqua-destilation is dropped using a 1cc disposable syringe and left for 5 minutes. Then add PBS and wait for 5 minutes. After that, it drops with hydrogen peroxidase block for 10 minutes. Dropped PBS twice for 5 minutes (the sauce is smoked slowly with tissue). Trypsin drops for 15 minutes and puts an incubator. Dropped with Ultra Violet Block for 5 minutes. Then rinsed with PBS and immediately dropped with OVGP primary antibody antibodies for 1 hour, after that washed with PBS twice for 5 minutes. Drop the Biotylated link (yellow) for 30 minutes. Then dripping PBS twice for 5 minutes. Streptavidin (Red) drops for 30 minutes and drops with PBS for 5 minutes. Dropped with chromagen DAB for 10 minutes. And PBS drops for 5 minutes. Then in drops of aqua-destilation for 5 minutes after that, it was stained with methylene green and flowed with water for 5 minutes (Schmidt, 2001).

2.3 OVGP1 Expression on Follicles

Histological preparations of ovarian organs were with xylol I, xylol II, absolute ethanol I, absolute ethanol II, stratified ethanol (90%, 80%, 70%, 30%), and distilled water for 1 x 5 minutes respectively, then washed with PBS pH 7.4 3 times. Furthermore, the preparations were mixed in 10 mM citrate buffer pH 6 and 1 mM EDTA pH 8 for 10-20 minutes at 90 oC then the slides were washed using distilled water. The next stage is the process of blocking tissue peroxidase using 3% H2O2 in methanol for 10 minutes, then washed with PBS 3 times. The next process is blocking the slide with 1% skim milk in PBS-tween for 30 minutes, then the slide is washed using PBS 3 times. The next slide is given with primary antibodies in a ratio of 1: 100 in 1% skim milk and PBS-tween. The slides are then stored at 4oC for approximately 24 hours. Then the slide was washed with PBS 3 times. The next process is the addition of secondary antibodies with a ratio of 1: 300 in PBS that is left for 1 hour, then the slides are washed with PBS 3 times (Hadi, 2015).

The histological preparations were then dropped by SA-HRP (Strep Avidin Horse Radish Peroxidase) in a ratio of 1: 500 in PBS and incubated for 45 minutes at room temperature. Then washed again with PBS pH 7.4 3 times, then dropped with DAB (Diamano Benzidine) and incubated for 30 minutes at room temperature. The slides were then washed again with PBS pH 7.4 3 times, then counterstaining the slides with Mayer Hematoxyler for 10 minutes. The preparations are then washed with running water for 10 minutes, then rinsed with distilled water and dried for about 1 night. The final stage is the mounting process by using the glass and then covered with a glass cover. Subsequent results were observed using a microscope with a magnification of 400x (Hadi, 2015).

3 RESULT AND DISCUSSION

3.1 OVGP1 Expression in Goat Follicles

McCauley *et al*., (2003) mention Epihelliacell OVGP1 Glycoprotein (EOGPs) in mice and pigs localized in the zona pellucida, perivitelline space, and plasma oocyte membranes taken from oviducts (in vivo) and embryos. In this study, based on immunohistochemical analysis shows that the expression of OVGP1 (Oviduct Specific Glycoprotein 1) in goat follicles is found in the external theca cells, internal theca, liquor follicular, granulosa, and zona pellucida. The results of OVGP1 expression in goat follicles can be seen in **Figure 1** below.

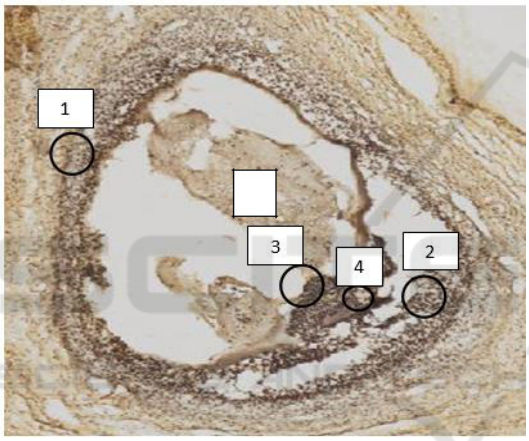

Figure 1. OVGP1 expression in Goat Follicles

Information:

1. Theca external cells
2. Internal theca cells
3. Granulosa cells
4. Zona Pelusida
5. Liquor follicular

3.2 OVGP1 Expression on Goat Oocytes

Based on the immunocytochemical analysis, OVGP1 expression on oocytes shows that there are cumulus oophorus complexes, zona pellucida, and perivitelline space. localized in part of the zona pellucida, perivitelline space, and plasma oocyte membrane is taken from the oviduct (in vivo) and the embryo. The results of OVGP1 expression in goat oocyte can be seen in **Figure 2** below.

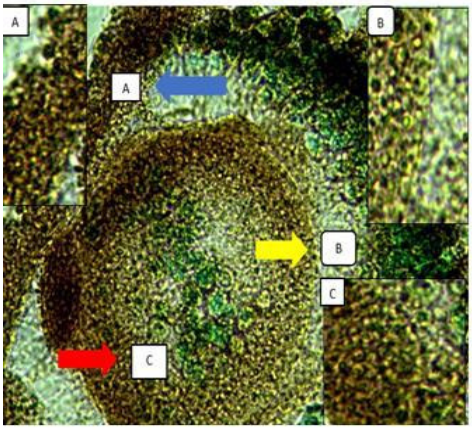

Figure 2. OVGP1 expression on Goat Oocytes

Information:

a) Cumulus oophorus Complex
b) Pelusida Zone
c) Perivitelin space

The OVGP1 expression of the results of this study is in the internal and external parts of the theca. OVGP1 which can be assumed also plays a role in the process of follicular maturation along with GDF-9 which results from the maturation process of this follicle that will produce estrogen synthesis. On the part of granulosa cells that are expressed in follicles and oocytes, this can play a role in the nourishment process of eggs during follicle ripening and maturation of oocytes from goats, whereas OVGP1 which is expressed in the zone of the pellucid and perivitelline space shows that OVGP1 will bind to TGF- β to inhibit polyspremia in the process of fertilization. In addition, OVGP1 together with GDF-9 late has been shown to play a role in the process of folliculogenesis and oocyte maturation. Based on the results of this study, it can be concluded that OVGP1 also plays a role in the process of follicular and oocyte maturation along with GDF-9 and TGF-β as well as zone and vitellin blockade. In addition to the role of growth factors that directly interact with OVGP1 as a receptor in the signaling process, there are also important roles of the FSH, LH and estrogen hormones that play an important role together with OVGP1. Where the increase in OVGP1 levels in the follicular phase is higher than in OVGP1 levels in the luteal phase. It can be concluded that OVGP1 in addition to playing a role in the process of fertilization, polyspermia blockade, early embryonic development, OVGP1 also plays an important role in the process of preovulation, ovulation, and oocyte maturation.

According to Buhi (2002), OVGP1 expression depends on the stage of the estrous cycle and is associated with circulating estrogen concentrations. Estrogen will stimulate OVGP1 expression. The synthesis of estrogen occurs from aromatization enzymes that produce androgen hormones whic are convertedto estradiol 17β under the influence of FSH (Liben, 2000). In the ovarian follicle, the hormone estrogen will be formed by granulosa cells through enzymatic reactions. The activity of these granulosa cells is related to the activity of the hormones FSH and estrogen. OVGP1 can indirectly increase some of the effects of the FSH related to the activity of GDF-9 aromatization in granulosa cells and the promotion of DNA synthesis in follicular cells (Laheri *et al*., 2017). OVGP1 expression is influenced by hormonal levels while still in the ovary, so that OVGP1 expression is related to hormonal influences (Buhi, 2002). OVGP1 expression in the zona pellucida indicates that OVGP1 will later bind to TGF-β which is TGF- this is a receptor of OVGP1 which will then cause complex bonding so as to increase defense of the zone and vitellin blockade so as to prevent polyspermia.

4 CONCLUSIONS

OVGP1 expression in goat follicles is expressed in the external theca cells, internal, granulosa, zona pellucida and liquor follicle while OVGP1 expression in oocytes is expressed in the cumulus oophorus complex, the zona pellucida, and perivitelline space.

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