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Profiling Glycoprotein of epithelial cell of oviduct at two-stages estrous cycle on Peranakan Ettawah (PE) Goat

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Abstract : Environmental condition inside the oviductal tube plays important roles for ovum viability after ovulation, sperms transport, fertilization, and early embryonic development. The composition of oviductal fluid derived from the estrogen-dependent activity of epithelial cell on its lumen is valuable in order to improve the in vitro fertilization (IVF) medium using additional specific glycoprotein of the oviduct epithelial cell secretion. The present investigation was carried out to find out whether the protein composition differs between the phases in the estrous cycle, both follicular and luteal phase and to determine composition of glycoprotein range 10-150 kDa from both phases. Electrophoresis SDS-PAGE was used to identify the oviductal glycoprotein derived from oviductal epithelial cell (OEC) in 12,5% gel concentration. Gel Documentation was used to identify varying intensity and molecular weight of each bands of the gels. Ten proteins of molecular weight in the range of 17– 74 kDa were expressed between the two phases, for sample of luteal 1 and follicular 1 expressed less than 10 proteins on the gel. Only one prominent bands corresponding to 67 kDa were observed in the OEC in every samples, and heavily stained proteins of 45 kDa, 50 kDa, 74 kDa and 98 kDa were present in some samples. In conclusion, oviductal secretions derived from OEC undergo alterations in an oestrus-dependent manner and these changes were supportive for the functional role of oviducts in PE reproductive tract.
Keywords : oviduct, glycoprotein profile, estrous cycle, goat.

Introduction

The female reproductive tract is a highly dynamic organ system. The oviduct in mammals undergo numerous sequential morphological changes during the oestrous cycle, driven by cyclic fluctuations in several reproductive hormones. Physiology of oviduct in mammals have been studied intensively for many years, whether old or new information has contributed to the understanding of fertilization and pre-implantation embryo development. In mammals, the oviduct is the site where the oocyte be fertilized and zygot starts to develop. The whole process happens within the lumen of the oviduct and uterus, with variation composition of fluid inside the lumen of both the oviduct and uterine (Coy and Yanagimachi, 2015).

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Oviductal fluid contains amino acids, proteins, simple carbohydrates and complex ions, fats and phospholipids. These components include metabolic subtract such lactic, pyruvic acid, amino acids and glucose that the level is different in uterine liquids and serum (Hugentobler et al. 2007). Volume and components of some proteins in oviductal liquids is changing during estrous cycles. Part of the complex of proteins in oviductal liquids is produced from transudate serum, however there is also a specific protein synthesis and secretion of oviduct epithelium, and the secretion of these proteins is regulated by hormonal changes, followed by an increase in biosynthesis of pre-ovulatory period (Buhi, 2002). During the oestrous cycles, the main function of oviduct on the reproduction process involves interaction with gametes. Therefore, it can be hypothesized that the regulation of proteins secretion in oviduct fluid based on the oestrous-oriented interacting with gametes. On the other hand, when the luteal phase, the amount of differential protein in the uterus can changes in the concentration and type. Knowledge of the normal proteins distribution of the reproductive tract, especially the oviduct, at each stage of the oestrous cycle becomes essential.

Somatic cells like granulosa cells and oviductal cells were reported to secrete proteins and growth factors besides removing inhibitory components from in vitro cell culture environment (Nandi et al., 1998). The metabolic cooperation between the oocytes and cumulus cells served an important nutritive role during oocyte maturation. Moreover, there were evidence that oviductal cell monolayer was found to be able to increase the developmental competence of oocytes and embryos of goat and sheep (Holm et al., 1994, Cognie et al., 2003)

McCauley et al., (2003) mentions Epihelliacell Oviductin Glycoprotein (EOGPs) localized in the zonapellucida, perivitelin space, and plasma membrane of oocytes extracted from oviduct (in vivo) and embryo. This suggests the possible role of EOGPs in fertilization and early embryonic development. Oviduct glycoproteins (OGP) in addition to being associated with the zonapellucida are also localized to the perivitellin space (Buhi, 2002). Its existence in theperivitellin space may be related to the mechanism of the polyspermi block ievitelin block.

Detection and analysis of proteins, synthesized and secreted from the oviduct epithelial cells find their distribution temporal and spatial of macro molecules and the differences in the distribution of proteins in various species. The identification of the protein profile on Peranakan Ettawah (PE) goat's oviduct has not been studied yet. Knowledge of the glycoprotein profile derived from epithelial cells of the oviduct at each stage of the oestrous cycle is essential when evaluating female reproductive tissues for evidence of its regulation during fertilization and embryogenesis.

Materials and methods

Sample preparation

Female reproductive organs derived from waste of Slaughterhouse Animal, Gadang, Malang, collected a maximum of 2 hours after the slaughtering process. For the preparation of the organ, tissues were washed twice using sterile PBS and placed in a petridish. Determination of the follicular and luteal phases was conducted by observing the dominant forms in ovarian macroscopically. The follicular phase marked by the formation of the dominant follicle in the ovary, meanwhile the luteal phase marked by the formation of the corpus luteum with minimum diameter of 0.5 mm.

Collection of oviductal epithelial cells for protein extraction

Ligated oviducts ipsilateral to the ovary at two stages of estrous cycle were washed several times in physiological saline and trimmed off the surrounding tissues. Oviductal cells were harvested by gentle stripping of the oviducts as per the procedure of Velazquez et al. (2000) using light scalpel. The protein extraction was used under protocol of DeRipro KIT for tissue protein extraction.

Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE)

In the present investigation, 12,5% SDS-PAGE was carried out with extracted protein from oviductal epithelial cells from follicular and lutheal phase of the estrous cycle. A medium range molecular weight marker set (GangNam Stain, Intron Biotech) was used for the molecular weight reference. The electrophoresis (Vertical SDS PAGE, Bio Rad) was done at constant voltage (50 V) at room temperature.

Detection of Protein by Coomassie Brilliant Blue Staining

After completing the electrophoresis, the gels were rinsed with distilled water for 2 min and stained with 0.5% Coomassie brilliant blue R-250 in 40% methanol and 10% acetic acid, at room temperature, for 2

hrs. The gels were then destained in a solution containing 40% methanol and 10% acetic acid until background became clear. Finally, the gels were washed with Milli-Q water and utilized for further analysis. The intensity and molecular weight of the protein bands in the gels were identified using gel documentation (Bio Rad, CA, USA). The band area was measured in pixels.

Results

In general, protein purification always begins with the stage of protein isolation (cell separation) to produce a crude extract called crude protein. What distinguishes the intracellular protein, there is the stage of cell destruction before protein isolation is done. Cell destruction can be done chemically, physically, and enzymatically. Through the method of electrophoresis SDS PAGE it can be seen whether the isolated protein is a protein with the desired molecular weight and whether there are non-target protein molecules. SDS-PAGE results can be seen in Figure 1.

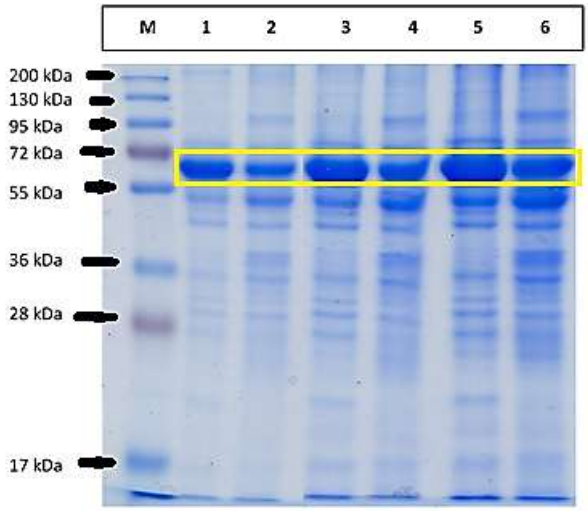

Figure 1.Differential protein expression pattern of oviduct epithelial cells during two stages of oestrous cycle. Well number 1, 3 and 5 belong to luteal group; well number 2, 4 and 6 belong to luteal group. M: Marker (GangNam Stain, Intron Biotech). Total crude protein volume in each well: 4ul, in a concentration 300mg/ml.

Lane and band analysis

Sampel No.											
Luteal 1		Folicular 1		Luteal 2		Folicular 2		Luteal 3		Folicular 3	
Band No.	Molecular Weight (kDa)	Band No.	Molecular Weight (kDa)	Band No.	Molecular Weight (kDa)	Band No.	Molecular Weight (kDa)	Band No.	Molecular Weight (kDa)	Band No.	Molecular Weight (kDa)
1	74,1	1	98,7	1	74,1	1	98,7	1	74,1	1	98,7
2	67,3	2	74,1	2	67,3	2	74,1	2	67,3	2	74,1
3	50,6	3	67,3	3	50,6	3	67,3	3	50,6	3	67,3
4	44,8	4	50,6	4	44,8	4	50,6	4	44,8	4	50,6
5	33,7	5	44,8	5	33,7	5	44,8	5	33,7	5	44,8
6	27,9	6	37,2	6	30,8	6	33,7	6	30,8	6	33,7
		7	33,7	7	28,9	7	37,2	7	28,9	7	37,2
		8	28,9	8	25,5	8	33,7	8	25,5	8	33,7
				9	20,0	9	28,9	9	20,0	9	28,9
				10	17,7	10	17,7	10	17,7	10	17,7

Gel Doc™ EZ imager. Exposure Time (sec) 0.322 used for auto-intensed band to automatically detected bands with high sensitivity. Molecular Weight Analysis using GangNam-stain standard with linear (semi-log) regression method. Regression Equation : y = -1.81 * x + 2.32; R-squared value: R^2 = 0.95

Total proteins extracted from oviductal fluid of PE goats harvested from abbatoir later be identified into two phases of the oestrous cycle. Polypeptides of the oviductal fluid belonging to the different stages of the cycle separated under reducing conditions were presented in Figure 1. The secretory protein profile revealed a repertoire of polypeptides represented in all the two stages analyzed. Most of the polypeptides showed identical expression of proteins in all the six samples tested. As loading of protein samples were normalized, differential bands varying in intensity were identified using GelDoc analyzer. Ten proteins of molecular weight in the range of 17– 74kDa were expressed between the two phases, for sample of luteal 1 and follicular 1 expressed less than 10 proteins on the gel. It could be noticed that several proteins were expressed only in the OEC belonging to follicular group.

It was observed that one prominent bands corresponding to 67 kDa were observed in the oviductalin each band,and heavily stained proteins of 34 kDa, 45 kDa, 50 kDa, 74 kDa and 98kDa were present in some samples. Similar protein profile of thick bands at these positions was noticed in our study and could be conceived that they were oviduct-derived proteins. It is also a common feature that secretory oviductal epithelial cells (OEC) produce a wide array of proteins that may comprise of growth factors, small peptides, cytokines, hormones and the like throughout the cycle (Woldesenbet and Newton, 2003). In this study, differential expression of oviductal fluid proteins was observed between the follicular and luteal phases of oviducts for someproteins of molecular weight of 37kDa and 98 kDa. Another protein in the range of 20kDa and the other in in the range of 17 – 28kDa were observed, but not specifically different between the two phases.

Discussion

It is worth mentioning here that the oviductal epithelium is capable to synthesis some proteins under estrogen control and released them under progesterone control. Similar differential expression of secretions of OEC were earlier demonstrated by other authors (Ulbrichet al., 2003 and Kadam et al., 2007) indicating that oviductal secretions at different stages of the oestrous cycle could have varying effects on the gametes during the course of the cycle. Severral studies have shown that proteins unique to the time of ovulation were secreted at the time of ovulation. In a 2D gel analysis study by Seytanoglu et al. (2008), 51 proteins were found to be unpregulated by 2-fold and 27 proteins downregulated when follicular and luteal stage oviduct fluid were compared. Acquisition of functional competence of gametes has been reported to be influenced by the oviductal fluid secretions belonging to different phases during the estrous cycle.

This study also observed the expression of few proteins in the oviducts belonging to non cycling group while they were not found in any phase of the cycling animals. Probably these might be members of inhibitory proteins or binding proteins that blocked the activity of essential oviductal proteins. All these changes suggested that oviductal secretions undergo alterations in an oestrus-dependent manner and these changes were supportive for the functional role of oviducts in reproduction.

During the oestrous cycle, main function of the cervix and the oviduct in the reproduction process involves interaction with gametes, which are present in the oestrous phase. These changes cause the micro environment (microenvironment) specified in the oviduct to the maturation of gametes, sperm capacitation, gamete and embryo transport, fertilization and early embryo cleavage. In the follicular phase, estrogen is important for epithelial differentiation and the development to maturation of cells and their secretory production of macro molecules. Therefore, the lumen environment consists of macromolecules and proteins derived from serum transudate or epithelial secretion. In the last decade it has been said that the oviduct and secretion oviduct plays an important role in various reproductive activities. Proteins produced to help increase the success of fertility, increased its concentration during proestrus and oestrus phase under the control of estrogen.

Oviduct physiology of mammals have been studied intensively for many years. All information, whether old or new information has contributed to the understanding of fertilization and preimplantation embryo development. In mammals, the oviduct is the site where oocyte and zygote fertilized and zygot development stage. Oocytes were released from the ovarian follicles will be caught by the infundibulum. Oocytes will be forwarded to the ampulla to fertilize with spermatozoa into a zygote. Zygote will develop during the past the isthmus of the oviduct. Later, the early stages of embryo development phase (4 to 8 cells) into the uterus so that implantation to form the placenta. The whole process happens within the lumen of the

oviduct and uterus, where the fluid is contained within the lumen of the oviduct and uterine (Coy and Yanagimachi, 2015).

The fluid in the oviduct of mammals give biologic environment for fertilization and early cleavage stage embryos. Irregularities in the oviduct fluid composition can be detrimental for these processes. According Buhi (2002), the oviduct of mammals significant changes in physical, morphological, and biochemical induced by endocrine during estrus cycles or menstruation. These changes cause the micro environment (microenvironment) specified in the oviduct to the maturation of gametes, sperm capacitation, gamete and embryo transport, fertilization and early embryo cleavage. This complex function dependent on the activities of both the secretory epithelium and ciliated and non-ciliated that covers oviduct mucosal. The function of these cells are regulated by ovarian steroids, estrogen and progesterone. During the follicular phase, estrogen is important for epithelial differentiation and the development and maturation of cells and their secretory production of macro molecules. Therefore, the lumen environment consists of macromolecules and proteins derived from serum transudate or epithelial secretion. In the last decade it has been said that the oviduct and its secretion plays an important role in various reproductive events and been identified its potential role in embryonic development in vitro (Pradeep et al., 2011).

Oviductin and heparin-like glycosaminoglycans (GAGs) of liquid oviduct has been demonstrated in pigs and cows participate in the modification of functional zonapellucida that occurs before fertilization and create a zone even become more resistant to dissolution enzymatic, penetration of sperm, as well as providing control against polispermi (Coy et al., 2008). Family glycoprotein identified on the liquid oviduct by name oviduct secretory glycoprotein (pOSP), oestrus-associated glycoprotein (EAP), oviductspecific, oestrus-associated glycoprotein (EGP), oviduct glycoprotein (sOP92), oviduktin, MUC-9, a glycoprotein GP 215 and oviduct -specific glycoprotein (OGP) or collectively referred to as the oviduct-specific, estrogen-dependent glycoproteins (OGPs). OGPs molecular weight and different carbohydrate content is released by the oviduct epithelial secretory all mammals except for mice and horses. OGPs expression differences in the infundibulum, ampulla and isthmus has also been demonstrated. OGPs expression apparently regulated by estrogen, or estrogen-related, but there are differences among the species. The same one universal character of OGPs is relation to the zonapellucida and perivitelin space of oocytes and embryos (Buhi, 2002), with the exception of the zonapellucida mice.

Conclusions

In conclusion, this finding suggests that the difference protein expressed in the SDS gel which are found in both folicullar and luteal phase could be important in regulating fertilization and embryogenesis on PE goat. Hopefully this recent study gives information of the protein markers in physiology expression in epithelial cell of oviduct of PE goat, to assist better understanding of its importance in tractus reproduction during follicular and luteal phase in order to maintain gamete transport, sperm and oocyte changes inside the oviductal environment. Moreover, the result from this study can be utilized to develop better strategies for increasing reproductive efficiency especially in domestic ruminants.

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