Interventions of Cetrorelix Acetate in Estrogen Beta Receptor Expression and Histopathology in Rats Oviduct

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Abstract: Ovarian hypofunction is pathologic conditions where is the ovary being abnormal. The abnormality of the ovary can be induced by the abnormality of the endocrine that regulates the development of the ovary such as, follicle-stimulating hormone (FSH) and luteinizing hormone LH. The production of FSH and LH in the pituitary is determined with the Gonadotropin hormone (GnRH) stimulation. Development of rat ovarian hypofunction models can be performed with the induction of cetrorelix acetate which has an antagonist effect of GnRH. This research was conduct to know the effect of induction of cetrorelix acetate on rat oviduct estrogen beta receptor expression and histopathology. The study used three groups of female rats (Wistar strain) 8-10 weeks old and 150-180 gram weight, each group consisting of six rats. The first group (control) without cetrorelix acetate, the second group treated with cetrorelix acetate 0.009 mg/kg BW and the third group treated with cetrorelix acetate 0.0135 mg/kg BW. Observations of estrogen beta receptor expressions (ERs β) are carried out with immunochemical methods, while observations of histopathological changes of oviduct carried out by Hematocsilin-Eosin (HE stain). The results obtained indicate a significant difference from the administration of the GnRH antagonists in the three treatment groups, among others, the largest reduction of the expression of the estrogen receptor of the ES β by 59.2%, as well as the thinning of the fallopian tubes and the reduced cilia. The conclusion of the study was Cetrorelix acetate as a GnRH antagonist capable of lowering the beta estrogen receptor expression and reducing the number of cilia as well as the viscosity of the wall lining of the fallopian tubes.

1 INTRODUCTION

The consumption need of protein from animals source especially for meat per capita in Indonesia for one day reaches 3.35 grams or 5.91%. This needs every year increases. The largest increase in consumption of livestock products experienced was in 2016 about 32.17% (Ditjen PKH, 2017). The increasing number of consumption of livestock products, especially meat, can cause an increase in import activities. One of the causes of the increase in beef import activities in Indonesia is the low reproduction performance of female cows in Indonesia and reproductive disorders. One of the most common examples of reproductive disorders in female cows is ovarian hypofunction.

Sutiyono et al. (2017) explain that ovarian hypofunction is a decrease in ovarian activity in producing oosit or ovum. Ovarian hypofunction is a pathologic condition caused by impaired secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Hermadi, 2015). Impaired secretion of FSH and LH can be caused by decreased secretion of Gonadotropin-Releasing Hormone (GnRH) by the hypothalamus. According to Wulandari (2013), GnRH functions to stimulate FSH and LH secretion. FSH functions are to stimulate follicular development and estrogen secretion, while LH functions for the maturation of de Graaf follicle and ovulation. The development and function of the reproductive organs are highly dependent on the secretion of FSH and LH in the

anterior pituitary controlled by GnRH in the hypothalamus. The low secretion of GnRH in the hypothalamus and low secretion of FSH-LH in the anterior pituitary will cause anestrus animals (Pemayun, 2010). One of the medicines that have an effect on GnRH antagonists is Cetrorelix acetate. This GnRH antagonist can cause a decrease in ovarian function by suppressing binding between GnRH and its receptors so it can inhibit the synthesis of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) (Wen et al., 2010). The inhibition of FSH and LH synthesis causes inhibition of folliculogenesis (Sharif et al., 2016). Delay in folliculogenesis can cause inhibition of estrogen hormone synthesis which results in decreased expression of estrogen receptors in tissues (Caldon, 2014).

Estrogen is a steroid hormone that has functions in many tissues in the body, including the oviduct. The primary function of estrogen is for tissue proliferation of the reproductive organs and other tissues related to the reproductive system. The morphology of oviduct epithelial cells is influenced by ovarian hormones, one of which is estrogen (Crow et al., 1994). GnRH antagonists that inhibit estrogen synthesis cause a decrease in the bonds between estrogens and its receptors, so it will make the receptors inactive and not expressed. According to Trisunuwati (2016), estrogens need estrogen receptors to carry out their functions. In the oviduct, estrogen hormone activity requires binding with receptors to stimulate epithelial cell proliferation, so the absence of estrogen receptors in the fallopian tubes can cause inhibition of ciliary formation in the fallopian tubes.

2 MATERIALS AND METHOD

The tools used include terumo® 1 cc syringes, terumo® 3 cc syringes, blades, surgical scissors, anatomical tweezers, serological tweezers, surgical boards, Petri dishes, and pins, microtomes, incubators, and optilab microscopes.

Materials used include rabbit feed (pellets) SP®, husks, and sufficient water, Phosphate Buffer Saline (PBS), formaldehyde, alcohol, xylol, 0.9% physiological NaCl, paraffin, Hematoxillin-Eosin stain, entellan, primary antibody ERS β brand abcam® (ab288), secondary antibody labeled peroxidase, normal Horse serum 2.5% brand abcam® (ab7484), hydrogen peroxide, methanol and chromogen diaminobenzidine tetrahydrochloride (DAB) brand abcam® (ab64238).

The rats were acclimatized for 7 days for adaptions, given rabbit feed and drinking water in an adlibitum. The group was divided into 3 groups which included a control group without administration of Cetrorelix acetate, the first treatment group (P1) with the administration of 0.009 mg/kg BW Cetrorelix acetate and the second treatment group (P2) with 0.0135 mg/kg BW of Cetrorelix acetate.

The vaginal swab preparations carried out by dipping the cotton bud in physiological NaCl than the rat placed in a dorsal lying position. The vaginal swab done by inserting a cotton bud in the vagina by rotating 360°, then cotton bud removed and swab on the slide. The slide allowed to dry and then fixed using alcohol. The preparations that have been fixed with alcohol and which have dried then are stained with Eosin Negrosin for 15 minutes, then rinsed with running water with a small flow of water and rinsed slowly. The results of vaginal swabs are observed under a microscope with a magnification of 100x and 400x to see vaginal cells. The vaginal swab was carried out before injected with the cetrorelix acetate to equalize the estrous cycle of the rat.

After the treatments, the rat was euatanated with cervical dislocation method (University of Melbourne Ethics and Animal Welfare Commission, 2016). The rat is vertically opened from the posterior abdomen to the thorax cavity. The oviduct organs are taken, then washed with physiological NaCl and collected in pots containing 10% Formaldehyde.

The ovary is processed to block paraffin and then continue with Haemotoxyline-eosin and immunohistochemical stain. The changes in the histopathological of the oviduct observed with the number of cilia and the thickness of the fallopian tube wall. The expression estrogen beta receptor was carried out at 40 times magnification and 5 visual fields (Le, 2005 and Okada et al., 2004). Estrogen receptors in the oviduct observed in all layers of cells. Expression of the estrogen beta receptor observed by calculating the average number of expressed cells using the ImmunoRatio application.

3 RESULT

The estrogen beta receptor expression in the oviduct observed with immunohistochemical methods (Table 1). This method will give a brown color to the target cell. These receptor expressions vary according to the stage of the ovarian cycle and peak in the middle of the cycle (Amso et al., 1994; Pollow

et al., 1981). Estrogen hormones in the oviduct are involved in the regulation of oviduct functions themselves, such as oviduct fluid formation and gamete transport (McDonell et al., 2002). Functionally, estrogen beta receptor (ER-β) functions in the growth of oviduct, regulation of protein content and expression of growth factors. Whereas ER-β functions in the process of gamete transportation.

Table 1. Expression of ERs β in rat injected with cetrorelix acetate

Group	Average ERs β expression (%)
Control group	54,01[c]
P1 group	32,30[b]
P2 group	22,03[a]

Note: a, b, c notations indicate a significant difference between one treatment and another.

Based on the estrogen beta receptor expression in the table above, it can be seen that the average ERs β expression in the treatment group (P1 and P2) decreased compared to the control group. This data also showed that the higher dose of cetrorelix acetate has lowest expression of ERs β in rat. The expression of β ERs can be seen in the Figure 1, 2 amd 3 below.
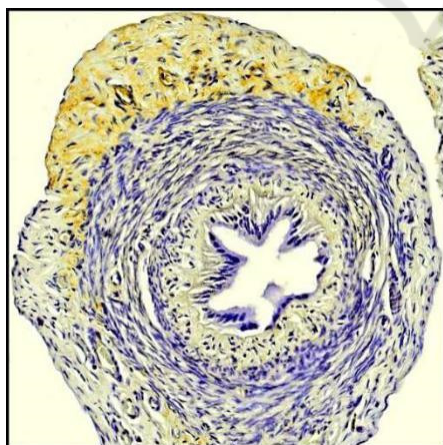

Figure 1. Expression of β ERs in rat oviduct of control group (40 times magnification, cross section).
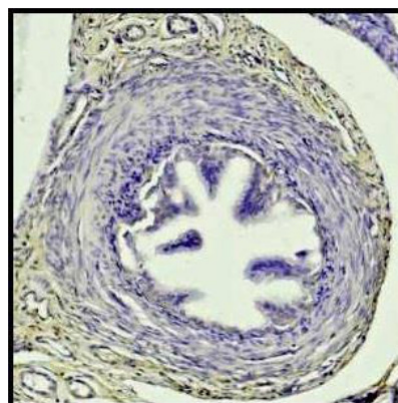

Figure 2. Expression of β ERs in rat oviduct of P1 group (40 times magnification, cross section).
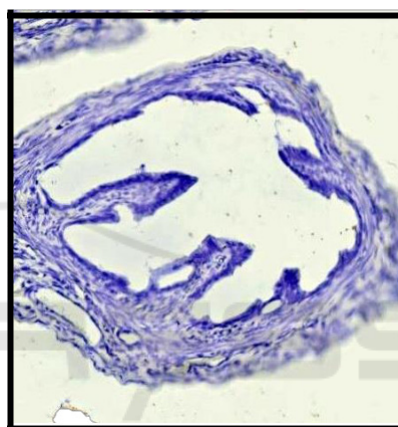

Figure 3. Expression of β ERs in rat oviduct of P2 group (40 times magnification, cross-section).

The results of the vaginal swab that conducted before euthanasia, showed that the rat was in the metestrus phase. The oviduct in the metestrus phase characterized by the thickening of the oviduct wall and the formation of cilia (Restall, 1966). An increasing number of ciliated cells and thick fallopian mucosal layers occur in the estrous and metestrus phases. Increased thickness of the fallopian tube lining is caused by an increase in the number of ciliated cells and secretory cells (Liputo, 2006). At the end of the metestrus phase, non-ciliary epithelial cells and ciliated epithelial cells undergo apoptosis, but most of the secretory granules remain in the secretory cells which then revert to structural changes and begin the process of ciliogenesis in the next phase (Kress and Morson, 2007).
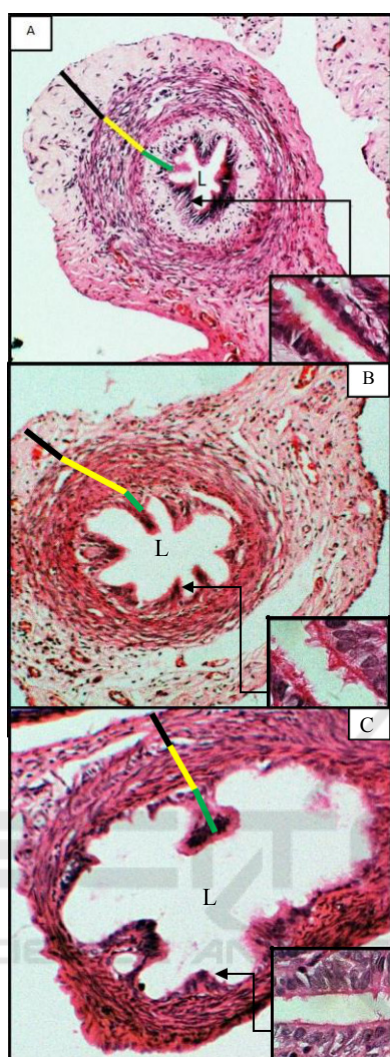
Figure 4. Histophatology of rat oviduct induced by cetrorelix acetate, A. Control group, B. P1 group, and C. P2 group. Haemotoxylin-eosin stain, 40 times magnification.

Note:　　tunica adventitia (Serosa);
　　— tunica media (Muscularis);　tunica
　　— intima (Mucosa);
　(L) : lumen

The control group showed thicken wall and many cilia (Figure 4.A), wich group P1 and P2 reduce in-wall thick and the number of cilia in the oviduct (Figure 4. B and C).

4 DISCUSSION

The expression of ER β decreased in the treatment group P1 injected Cetrorelix acetate 0.009 mg/kg

BW and P2 injected Cetrorelix acetate 0.0135 mg/kg BW. Decreased expression of β estrogen receptors caused by injection of Cetrorelix acetate as a Gonadotropin-Releasing Hormone (GnRH) antagonist occurs because the cetrorelix acetate competes with GnRH to bind to membrane receptors on pituitary cells and control the release of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH), thereby delaying the LH surge and ovulation (Rodney, 2013). Induction of Cetrorelix acetate which is a GnRH antagonist causes inhibition of estrogen synthesis that occurs during the process of folliculogenesis it makes a decrease of estrogen production. This decrease causes a decreased binding of estrogen and its receptor so the receptors are inactive and not expressed. These results can be concluded that the injection of Cetrorelix acetate can decrease the estrogen receptor expression in the oviduct.

The reduced number of cilia and the thickness of the oviduct wall caused by the injected by Cetrorelix acetate, it caused by the suppressed secretion of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and estrogen (Griesinger et al., 2005). Decreased levels of FSH and LH can inhibit folliculogenesis and ovulation (Cooke et al., 1998; Hafizuddin et al., 2012). Inhibited folliculogenesis by injection of cetrorelix acetate can cause impaired estrogen hormone synthesis. Impaired synthesis of estrogen hormones can affect the development of reproductive organs including oviduct wall.

The function of estrogen is for epithelial cell proliferation, secretion, and ciliogenesis (Verhage et al., 1979; Donnez et al., 1985). Ciliogenic activity and secretion are caused by estrogen which acts through estrogen receptors on oviduct epithelial cells (Lauschová, 1999; Listy and Chakravarti, 2011). The mechanism of the hormone estrogen which can affect the thickness of the oviduct can be explained through estrogen activity in the cells making up the oviduct. Estrogen activity in cells begins after estrogen bonds in the cytosol. The estrogen and receptor complex further diffuses into the cell nucleus and attaches to DNA. The estrogen-receptor complex binding with DNA induces the synthesis and expression of mRNA in the form of protein synthesis thereby increasing target cell activity, which is indicated by cell proliferation (Johnson and Everitt, 1984).

Increased thickness of the oviduct mucosal wall caused by an increase in the number of cells making up the fallopian tissue. Increasing the number of cells both secretory epithelial cells and ciliary epithelium can cause the oviduct mucosal layer to

get thicker. The thickness of the oviduct mucosal wall affects individual fertility (Umami et. Al, 2014). This is consistent with the statement of Crow et al. (1994) regarding the morphology of oviduct epithelial cells affected by estrogen, which act through their receptors, where the hormone estrogen causes mucosal glandular tissue to proliferate and increases the number of ciliated epithelial cells.

5 CONCLUSION

Injection of Cetrorelix acetate as GnRH antagonist in the rat can reduce the expression of estrogen beta receptor, decrease wall thickness and cilia in rat oviduct with the best doses was 0.0135 mg/kg BW.

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