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Evaluation of Estrogen Alpha Receptor (ER-A) and Progesterone-A Receptor (PR-A) Expression in the Oviducts and Uterus of Indonesian Local Rabbits after Induced by Several Method of Pseudo-Pregnancy

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Abstract

The objective of this study was to determine the expression of ER-A and PR-A in the oviduct and uterus of pseudo-pregnant rabbits after induced by several induction methods of pseudo-pregnancy. This study used 30 local rabbits, aged 1 - 1.5 years, body weight of 2.8 - 3.0 kg and clinically healthy. Rabbits were divided into 6 groups (n = 5), namely: K1 (negative control, given 1.5 mL physiological NaCl injection), K2 (positive control, given 100 IU eCG injection and mated with male rabbits, followed by 75 IU hCG injection 3 days later), K3 (given 100 IU eCG injection followed by injection of 75 IU hCG 3 days later), K4 (artificial copulation was performed by inserting a 1 cm long cotton bud into the rabbit's vagina), K5 (ovulation induction by injection of 50 IU hCG) and K6 (ovulation induction by injection of 5 g GnRH). On the 8th day after treatment, rabbits were euthanized and their oviduct and uterus organs were taken for histological preparation and stained using the immunohistochemical staining. The expression of ER-A and PR-A was valued using the intensity score and analyzed using the Kruskal-Wallis test followed the Mann Whitney U test. The expression ER-A in the infundibulum, ampullary and isthmus of K1 was significantly different ($p < 0.05$) from K2 - K6 rabbits. The ER-A expression in the endometrium of uterine horn of K1 and K3 was significantly different with K2, K4, K5 and K6 rabbits ($p < 0.05$). Furthermore, PR-A expression in oviducts of K1 and K3 was lower than that in K2, K3, K5 and K6 ($p < 0.05$) and the expression in the endometrium, uterine glands and myometrium of K2 - K6 was higher than K1 rabbits ($p < 0.05$). In conclusion, the hormonal induction could be used to induce pseudopregnancy in local rabbits based on the ER-A and PR-A expressions in oviduct and uterus.

Keywords: Pseudo-pregnancy, ER-A, eCG and hCG hormones, Local rabbits, PR-A

Introduction

Pseudo-pregnant rabbits are animal models commonly used to study reproductive endocrinology [1], endometriosis therapy [2], and ovarian transplantation as a source of oocytes [3]. Several hormones are thought to play an important role in the development and maintenance of pseudo-pregnant rabbits [4]. Pseudo-pregnancy generally occurs in adult rabbits and is characterized by the preservation of the corpus luteum in the absence of a fetus. In adult rabbits, there are several mature follicles and a corpus luteum in the ovarian parenchyma. This indicates that the rabbit uterus is under the influence of estrogen and progesterone [5].

The concentration of progesterone in pseudo-pregnant rabbits increased from day 4 to day 5, and reached its peak on day 11, then slowly decreased and reached basal levels on day 19 to day 20. At the same time, the concentration of the hormone estrogen as measured on the 5th day fluctuated between 0 - 140 pg/mL with slightly higher estradiol content than estrone. However, changes in estrogen levels during pseudo-pregnancy cannot be known with certainty [6].

Estrogen and progesterone will have physiological effects if these hormones interact with their receptors on target organ tissues, such as the reproductive organs [7]. These receptors include the estrogen receptor (ER) and the progesterone receptor (PR). One of the isoforms of ER besides ER beta (ERβ) is estrogen receptor alpha (ER-A) which plays an important role in the physiological functioning of the female reproductive tract. The presence of ER-A is found in the ovaries, mammary glands, uterus, testes, pituitary glands, hippocampus, kidneys, epidermis and adrenal glands (Rai and Jeswar citing to [8]). Furthermore, the progesterone receptor also has 2 isoforms, namely PR-A and PR-B [9]. The presence of these receptors in the tissue of an organ can be detected using the immunohistochemical (IHC) technique. This IHC technique can be used to detect the occurrence of bonds between the antigen in the tissue and the exogenous antibody [10].

The localization of PR was reported in the uterus of pseudo-pregnant rats and rabbits [11-13]. Additionally, detection of the distribution of ER-A and PR in the oviduct of cattle in the follicular and luteal phases using the IHC technique has been carried out by Saruhan *et al*. [10]. The IHC technique has also been applied to detecting the distribution and expression of PR-A and PR-B in cow oviducts during the estrus cycle and pregnancy phase by Saint-Dizier *et al*. [14]. However, the comparison of ER-A and PR-A expression in the uterus and oviduct of pseudo-pregnant rabbits induced by several methods of pseudo-pregnancy in the Indonesian local rabbits has not been reported.

Induction of pseudo-pregnancy by several methods resulted in different concentrations of progesterone with the highest concentration being obtained by the induction method with hCG or a combination of eCG and hCG ($p < 0.05$), while the concentration of estrogen showed no significant difference ($p > 0.05$) on the days 2, 4, 6 and 8 post-induction [15]. Changes in the concentration of reproductive hormones (progesterone and estrogen) that affect the uterus of pseudo-pregnant rabbits can be detected based on the presence of receptors for these 2 hormones. This study aimed to evaluate the expression of ER-A and PR-A in the oviduct and uterus of pseudo-pregnant rabbits induced by several methods of pseudo-pregnancy.

Materials and methods

Ethical clearance

This research was conducted at the Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh. This study was carried based on the ethical clearance certificate from the Research Animal Ethics Commission, Faculty of Veterinary Medicine, Universitas Syiah Kuala number 101/KEPH/V/2021.

Animals and treatment

This study used 30 local breed rabbits, aged 1 - 1.5 years, that were clinically healthy, with body weights of 2.8 - 3.0 kg. Prior to induction, each rabbit was placed in an individual cage for 3 weeks and received sunlight during the day and electric light at night. Rabbits were divided into 6 treatment groups (n = 5), namely K1 (negative control, given 1.5 mL physiological NaCl injection); K2 (positive control, given intramuscular injection of 100 IU eCG [Folligon, BV Boxmer, Holland] and mated with male rabbits, followed by intravenous injection of 75 IU hCG 3 days later [Chorulon, BV Boxmer, Holland] as directed by Schlegel *et al*. [16]); K3 (given intramuscular injection of 100 IU eCG [Folligon, BV Boxmer, Holland] followed by intravenous injection of 75 IU hCG 3 days later [Chorulon, BV Boxmer, Holland]) as directed by Schlegel *et al*. [16]); K4 (artificial copulation by inserting a 1 cm long cotton bud into the rabbit's vagina at 5 AM according to the instructions of Sumarmin *et al*. [3]); K5 (ovulation induction by intramuscular injection of 50 IU hCG [Chorulon, BV Boxmer, Holland] as directed by Abd-Elkareem [13]; and K6 (ovulation induction by intravenous injection of 5 g GnRH [Fertagyl, BV Boxmer, Holland] as directed by Viudes-de-Castro *et al*. [17]). Day 0 was the day when physiological NaCl was given to K1, mating was carried out for K2, hCG was injected to K3, copulation was induced to K4, hCG was injected to K5, and GnRH was injected to K6.

Organs sampling and histological preparation

On the 8th day after treatment, all rabbits were euthanized. Female reproductive tracts of rabbits which include oviducts and uterus were collected after laparotomy procedure and immediately fixed in 10 % neutral buffered formalin (NBF). After the fixation process, the oviducts and uterus (dexter et sinister) were transferred to 70 % ethanol as stopping point until histological preparations were made.

Histological preparation procedure refers to Kiernan [18]. The sample of oviducts and uterus were dehydrated in graded series of ethanol namely 80, 90, 95 %, and absolute, cleared in xylene solution, and infiltrated using liquid, and embedded in the paraffin. The paraffinized tissue was cut at 3 µm-thick sections

using a rotary manual microtome (Leica RM2235, Leica Biosystem, Nusslogh GmbH, Germany) and placed on clean surface of poly L-Lysine coated slides (Biogear[â], Biogear Scientific). All tissue slides were dried and stained with IHC staining using the avidin biotin peroxidase complex (ABC method) method.

Immunohistochemical staining

The procedure for IHC staining using the ABC method was performed according to the manual procedure of mouse and rabbit specific HRP/DAB (ABC) detection IHC kit (Invitrogen®, Thermo Scientific, USA). Prior to IHC staining, all slides were dewaxed by immersing in xylene solution and rehydrated in absolute, 95, 90, 80 and 70 % ethanol. The IHC staining was started by blocking endogenous peroxidase using a hydrogen peroxidase (H2O2) block solution and rinsed with PBS. The slides were then dripped with protein block, incubated, and washed with PBS. Next, the slides were incubated with primary antibodies (progesterone receptor antibody hPRa 2 and estrogen receptor/ERα) with a 1:200 dilution for both antibodies and then rinsed with PBS. The next step was incubation with biotinylated goat anti-polyvalent (secondary antibody), washed with PBS, incubation with streptavidin peroxidase, and then washed with PBS. Next, the slide was incubated with diaminobenzidine (DAB) chromogen for 1 - 10 min to visualization of the staining results. Positive results (immunoreactivity) were indicated by brown color with various levels of intensity. Furthermore, slides were counterstained using Mayer's hematoxylin solution, dehydrated in ethanol, cleared in xylene, and mounted with cover slips using Entellan® (Merck, Germany).

Observation of staining results

In this study, observations and identification of staining results were carried out using the intensity score (IS) method as described in **Table 1**. Intensity score is the scoring based on the intensity of immunoreactivity of ERα and PR-A on different parts of the tissue according to the IS method applied by Mudduwa [19].

Table 1 The IS method which applied for interpretation of ERα and PR-A expression in the oviduct and uterus of local rabbits.

Score	Expression level
0	No expression (negative)
1	Weak
2	Moderate
3	Strong

Data analysis

Data of the distribution of ER-A and PR-A in the oviducts and uterus was analyzed descriptively and presented in histological images. The expression of ERα and PR-A data obtained from IS in the oviducts and uterus tissue was analyzed using the non-parametric Kruskal-Wallis test and the Mann-Whitney U test [20].

Results and discussion

In the rabbit oviduct and uterine tissues, the presence of both hormone receptors (ER-A and PR-A) showed a variable distribution. Visualization of ER-A and PR-A immunoreactivity in the uteruses and oviducts was observed in the nuclei of cells, connective tissues and smooth muscle fibers. Observation of the distribution of ER-A and PR-A in the oviduct was focused on the infundibulum, ampulla and isthmus, namely the lamina epithelial, lamina propria and muscularis layers. In the uterine horn tissue, observations of the distribution of the 2 receptors were carried out on the endometrial layer, uterine glands and myometrium.

Distribution and expression of ER-A in the oviduct and uterus of the local rabbits

Distribution of ER-A in oviduct tissue of 6 treatment groups was found in the lamina epithelium, lamina propria and lamina muscularis of infundibulum, ampulla and isthmus (**Figure 1**). Previous studies also reported that distribution of ER-A was found in the oviducts, namely in the lamina epithelial layer, stroma and muscle cells of lamina muscularis of rat oviducts [21]. Distribution of ER-A based on immunoreactivity in the infundibulum, ampullary and oviductal isthmus of K1 rabbits was less than that of K2 - K6 rabbits, both in lamina epithelia and in lamina propria as well as in lamina muscularis. Difference

in distribution of these receptors is thought to be due to effect of hormonal induction given in K2, K3, K5 and K6. Similar distribution also found in oviduct tissue of rabbits induced by artificial copulation (K4). Abundant distribution of ER-A in K2 - K6 groups indicated an increase in concentration of estrogen in oviducts of both groups of rabbits induced by hormonal and artificial copulation. Additionally, distribution of ER-A was also found in infundibulum and ampulla of negative control group (K1), while in isthmus distribution of these receptors was not found. Saruhan *et al*. [10] reported that distribution of ERα was mostly found in lamina epithelium of infundibulum, ampulla and isthmus of oviduct tissue in cattle during follicular and luteal phases. According to Li *et al*. [22], estrogen functions to assist ovum transportation to the ampulla so that fertilization can occur. It has been found that estrogen affect length of cilia in oviductal lumen, but do not affect epithelial cell proliferation.

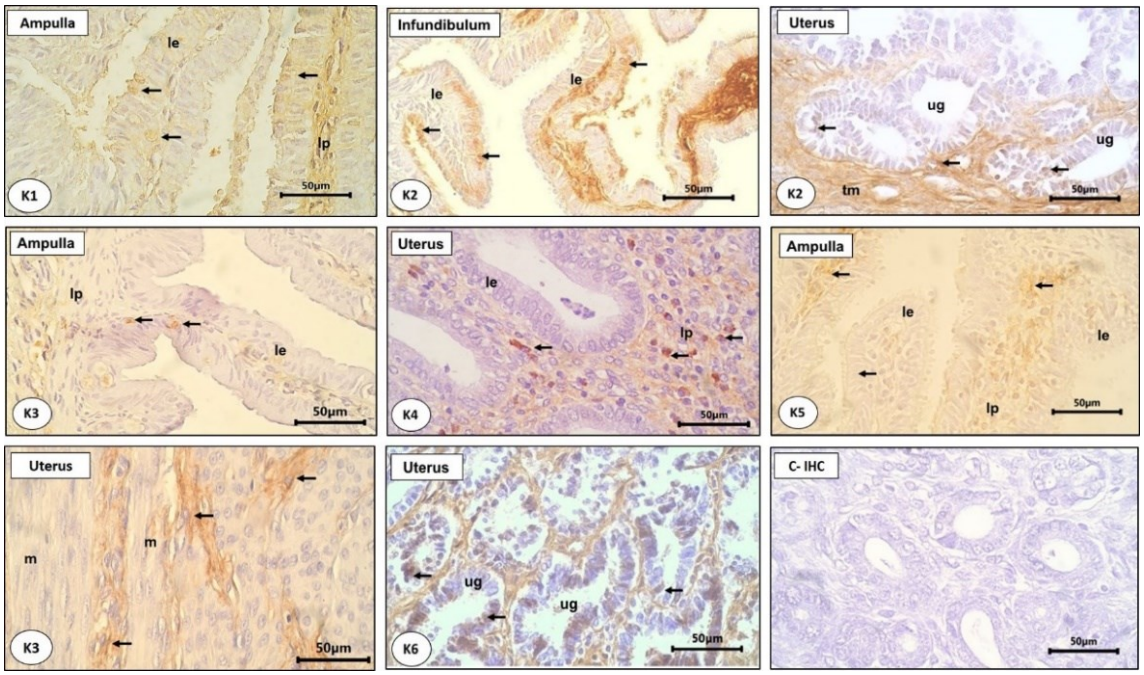

Figure 1 Distribution of ER-A in oviduct and uterus tissue of 6 treatments of pseudopregnancy in rabbit (K1 - K6). Immunoreactivity of ERα was found in ampulla, infundibulum and isthmus of oviduct (arrows). ER-A also immunoreactive in endometrium, myometrium and uterine glands of uterine tissue (arrows). Lamina epithelium (lm), lamina propria (lp), uterine gland (ug) and myometrium (m). C-IHC: Negative control of IHC staining. IHC staining, ABC method. Bar scale: 50 µm.

Differences in ER-A expression in the oviducts (infundibulum, ampulla and isthmus) are presented in **Table 2**. Expression of ER-A in oviducts K3 - K6 rabbit induced into pseudo-pregnancy was similar to expression of that receptor in oviducts tissue of K2 (positive control). According to Robinson *et al*. [23] and Wang *et al*. [24], ER-A expression in lamina epithelium increased during estrus phase and also during estrus phase and mid-luteal phase. According to Avila *et al*. [25], ER-A will be expressed in female reproductive tract, especially in lamina epithelium. Presence of ER-A in lamina propria of oviduct functions to regulate activity of ciliated epithelial cells found on mucosal surface of oviduct tract.

Table 2 Expression of ER-A in oviducts tissue of K1 - K6 local rabbit.

Oviduct parts	Layers	Treatment groups					
		K1	**K2**	**K3**	**K4**	**K5**	**K6**
Infundibulum	Epithelium	1.30 ± 0.67^{a}	2.80 ± 0.42^{b}	2.10 ± 1.10^{a}	2.40 ± 0.96^{b}	2.00 ± 0.94^{a}	1.00 ± 1.33^{a}
	Propria	2.40 ± 0.84^{a}	3.00 ± 0.00^{b}	2.40 ± 0.96^{a}	3.00 ± 0.00^{b}	2.60 ± 0.84^{a}	2.40 ± 0.51^{a}
	Muscularis	2.30 ± 0.82	2.90 ± 0.31	2.10 ± 0.87	2.90 ± 0.31	2.20 ± 0.78	2.80 ± 0.42
	Serosa	2.70 ± 0.67^{ab}	3.00 ± 0.00^{a}	2.40 ± 0.84^{b}	2.10 ± 0.99^{a}	2.50 ± 0.52^{b}	3.00 ± 0.00^{a}
Ampulla	Epithelia	1.60 ± 1.34	2.60 ± 0.51	2.00 ± 1.15	2.30 ± 0.67	2.30 ± 0.94	1.60 ± 1.42
	Propria	1.50 ± 1.35	2.50 ± 0.52	2.30 ± 0.94	2.90 ± 0.31	2.80 ± 0.42	2.20 ± 0.78
	Muscularis	1.90 ± 1.19^{ac}	1.60 ± 0.84^{a}	2.60 ± 0.51^{bc}	2.80 ± 0.42^{b}	2.00 ± 0.94^{ac}	2.60 ± 0.51^{bc}
	Serosa	1.80 ± 0.91^{a}	3.00 ± 0.00^{b}	2.10 ± 0.99^{ac}	2.60 ± 0.51^{c}	2.70 ± 0.48^{bc}	2.80 ± 0.42^{bc}
Isthmus	Epithelia	1.10 ± 0.87	2.50 ± 0.70	0.60 ± 0.84	1.40 ± 0.69	1.80 ± 1.31	1.10 ± 1.28
	Propria	1.50 ± 1.26	1.90 ± 0.73	1.50 ± 1.08	2.00 ± 0.66	1.40 ± 1.26	2.20 ± 0.78
	Muscularis	1.50 ± 1.35^{a}	2.70 ± 0.48^{b}	1.90 ± 0.99^{a}	2.30 ± 0.94^{ac}	$1.40 \pm 1,07^{a}$	2.80 ± 0.42^{bc}
	Serosa	2.00 ± 0.81^{a}	3.00 ± 0.00^{b}	1.90 ± 0.99^{a}	2.10 ± 0.87^{a}	2.60 ± 0.51^{a}	3.00 ± 0.00^{b}

Note: Different superscripts on the same line show significant difference ($p < 0.05$). K1: Negative control (physiological NaCl injection), K2: Positive control (100 IU eCG, mated, 75 IU hCG), K3: 100 IU eCG, 75 IU hCG, K4: Artificial copulation, K5: 50 IU hCG and K6: 5 g GnRH.

Another function of ER-A is to increase size of cilia in epithelial cells of oviduct, but do not affect epithelial cell proliferation. Because of function of estrogen which is mediated by ER-A, ovum can travel to ampulla and then fertilization occurs. After binding to hormone estrogen, additional functions of ER-A in oviduct are to increase ciliary activity, stimulate smooth muscle activity and facilitate embryo transport [22].

Immunoreactivity of ER-A was observed in all 3 parts of the oviduct (infundibulum, ampulla and isthmus) of K1 - K6 rabbits with stronger ERα expression found in oviduct of K2 - K6 rabbits. Expression of ER-A in lamina muscularis of infundibulum was significantly different between K1 and K2, K4, K6 rabbits ($p < 0.05$) but not significantly different between K1 and K3, K5 rabbits ($p > 0.05$). In ampulla, there were also differences in expression ER-A in lamina muscularis between K1, K2, K5 and K3, K4, K6 rabbits ($p < 0.05$). In lamina epithelium of isthmus, there were also differences in ER-A expression ($p < 0.05$) between rabbits K1 vs K2 and rabbits K2 vs K5 (**Table 2**). Based on the differences in ER-A expression in lamina muscularis and lamina epithelia of 3 parts of oviduct, it can be inferred that induction of pseudo-pregnancy increases secretion of estrogen from ovaries which affects function of the oviduct. This is presumably because concentration of estrogen in pseudo-pregnant rabbits fluctuates between 32 to 49 pg/mL and slowly increases in late period of pseudo-pregnancy [26]. According to Caillol *et al*. [6], concentration of estrogen in pseudo-pregnant rabbits fluctuated from 0 to 140 pg/mL and there was no definite change in estrogen levels during pseudo-pregnancy. Syafruddin *et al*. [15] reported a fluctuating increase in estrogen in local rabbits induced by several pseudo-pregnancy induction methods. Expression of ER-A in oviduct indicates role of estrogen in regulating activity of ciliated epithelial cells lining the mucosa of oviduct. Other functions of estrogen according to Kenngott *et al*. [27] is secreting high levels of protein in oviduct during the follicular phase.

Observations on location of ER-A distribution in uterine horns of K1 - K6 rabbits focused on endometrium, uterine glands and myometrium (**Figure 1**). Distribution of ER-A in uterine tissue of K1 - K6 rabbits was detected in endometrial layers, namely lamina epithelium, lamina muscularis and uterine glands. Distribution of ER-A expression at the same location was also reported by Winuthayanon *et al*. [28], in which ER-A was also expressed in luminal epithelial lining mucosa of uterine, glandular epithelium, stroma and myometrium of uterus in mice. This is in accordance with Lind [29], that ER-A is a specific receptor that allows for estrogen to physiologically effect in target organs. In this present study, expression of ER-A with a very strong intensity was found in the uterus of rabbits K2 - K6, while in uterus of K1 rabbits, distribution and expression of ER-A was not found. Furthermore, the ER-A receptor was also

immunoreactive in the uterine glands of rabbits K2 - K6, but in K1 rabbits it was less distributed with weak expression. This is presumably due to low concentration of estrogen in uterine glands of K1 rabbits and is related to the presence and expression of hormone receptors in uterine tissue of these rabbits.

Based on results of intensity score, differences in expression of ER-A were found in each uterine layer of K1 - K6 rabbits (**Table 3**). Expressions of immunoreactive ER-A in epithelial cells of the uterine horns were significantly different between K1, K3 and K2, K4, K5 and K6 rabbits ($p < 0.05$). Furthermore, differences in expression were also observed in myometrial layer between K1 and K2 - K6 rabbits ($p < 0.05$). In addition, in uterine glands there was a significant difference in ER-A expression ($p < 0.05$) between K1, K4 and K2, K3, K5, K6 rabbits. Differences of ER-A in each uterine layers of rabbits induced by several pseudo-pregnancy methods indicates that uterine tissue is highly responsive to hormonal change. According to Sitasiwi [30], endometrial layer consisting of lamina epithelium, lamina propria and uterine glands is responsive to changes in reproductive hormones. In the present study, it can be proven that there is an increase role of estrogen in uterine tissue which is shown by the presence of ER-A immunoreactivity which is widely distributed in uterine layers of K2, K4, K5 and K6 rabbits. Kuiper *et al*. [31], stated that the main function of ER-A in uterus is to mediate effects of estrogen.

Table 3 Expression of ER-A in the uterine tissue of K1 - K6 local rabbit.

Uterine layers	Treatment groups					
	K1	**K2**	**K3**	**K4**	**K5**	**K6**
Endometrium	1.20 ± 1.54^{a}	2.40 ± 0.84^{ac}	1.30 ± 1.25^{a}	2.90 ± 0.31^{bc}	2.80 ± 0.42^{bc}	2.70 ± 0.48^{bc}
Uterine glands	0.80 ± 1.22^{a}	2.60 ± 0.84^{d}	2.80 ± 0.42^{d}	0.60 ± 1.07^{a}	1.10 ± 1.37^{bc}	2.10 ± 1.19^{c}
Myometrium	1.30 ± 0.48^{a}	2.80 ± 0.42^{bd}	2.00 ± 0.67^{c}	2.50 ± 0.84^{bcd}	2.20 ± 0.91^{bc}	3.00 ± 0.00^{d}
Perimetrium	0.90 ± 0.56^{a}	2.80 ± 0.42^{b}	3.00 ± 0.00^{b}	3.00 ± 0.00^{b}	2.70 ± 0.67^{b}	3.00 ± 0.00^{b}

Note: Different superscripts on the same line show significant difference ($p < 0.05$). K1: Negative control (physiological NaCl injection), K2: Positive control (100 IU eCG, mated, 75 IU hCG), K3: 100 IU eCG, 75 IU hCG, K4: Artificial copulation, K5: 50 IU hCG and K6: 5 g GnRH.

Distribution and expression of PR-A in oviduct and uterus of local rabbit

PR-A distribution was found in lamina epithelium, lamina propria and lamina muscularis in oviduct tissues of K1 - K6 rabbits (**Figure 2**), but in oviduct tissues of K1 (negative control) rabbits, this receptor was found in small amounts in lamina epithelial of infundibulum, ampulla and isthmus. It is suspected that in K1 rabbits that were not induced by pseudo-pregnancy methods and not mated had much lower progesterone than estrogen concentrations.

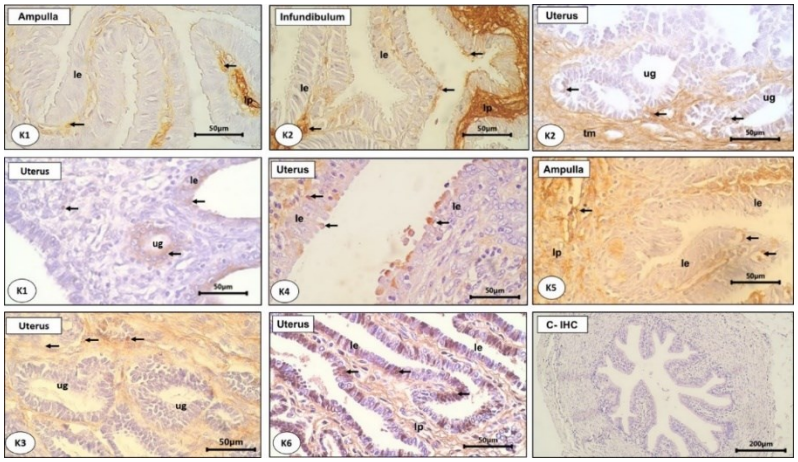

Figure 2 Distribution of PR-A in oviduct and uterus tissue of 6 treatments of pseudopregnancy in rabbit (K1 - K6). Immunoreactivity of PR-A was found in ampulla, infundibulum and isthmus of oviduct (arrows). PR-A also immunoreactive in the endometrium, myometrium and uterine glands of uterine tissue (arrows). Lamina epithelium (lm), lamina propria (lp), uterine gland (ug) and myometrium (m). C-IHC: Negative control of IHC staining. IHC staining, ABC method. Bar scale: 50 µm.

Expression of PR-A in isthmus lamina epithelium and lamina propria of K1 - K6 rabbits are presented in **Table 4**. Different distributions of PR-A were found in 3 parts of the oviduct. The most distribution of receptors and very strong expressions were found in lamina propria infundibulum and ampulla, whereas in isthmus the distribution was less with a weaker expression. Additionally, PR-A expression in K1 and K4 rabbit oviducts was lower than in K2, K3, K5 and K6 oviducts ($p < 0.05$). Peiro *et al*. [32], also conducted research on New Zealand rabbits and found that PR-A was expressed in ampulla to isthmus and there was no difference in its expression between oviduct tissues.

Table 4 Expression of PR-A in oviducts tissue of K1 - K6 local rabbit.

Oviduct parts	Layers	Treatment groups					
		K1	**K2**	**K3**	**K4**	**K5**	**K6**
Infundibulum	Epithelium	2.00 ± 1.05^{ab}	2.40 ± 0.69^{a}	2.60 ± 0.96^{a}	1.90 ± 1.10^{b}	2.70 ± 0.48^{ac}	3.00 ± 0.00^{c}
	Propria	2.70 ± 0.67	2.90 ± 0.31	2.70 ± 0.48	3.00 ± 0.00	2.70 ± 0.94	3.00 ± 0.00
	Muscularis	2.10 ± 0.87^{a}	2.80 ± 0.42^{bc}	2.40 ± 0.69^{ad}	2.90 ± 0.31^{cd}	2.20 ± 0.78^{a}	3.00 ± 0.00^{c}
	Serosa	2.50 ± 0.78	3.00 ± 0.00	2.50 ± 0.70	2.50 ± 0.84	2.90 ± 0.31	3.00 ± 0.00
Ampula	Epithelium	1.60 ± 1.17	2.80 ± 0.42	1.10 ± 0.87	2.20 ± 1.13	2.30 ± 1.05	2.80 ± 0.42
	Propria	1.60 ± 1.17^{a}	2.60 ± 0.69^{bc}	2.50 ± 0.70^{ac}	3.00 ± 0.00^{b}	3.00 ± 0.00^{b}	3.00 ± 0.00^{b}
	Muscularis	1.50 ± 1.17^{a}	2.50 ± 0.52^{c}	1.90 ± 1.10^{ac}	2.60 ± 0.69^{bc}	2.60 ± 0.51^{c}	3.00 ± 0.00^{b}
	Serosa	2.50 ± 0.70	2.80 ± 0.42	2.8 ± 0.63	2.60 ± 0.69	2.80 ± 0.42	3.00 ± 0.00
Isthmus	Epithelium	0.70 ± 1.05^{a}	2.50 ± 0.70^{b}	0.80 ± 1.22^{a}	1.40 ± 0.96^{a}	1.30 ± 0.94^{a}	1.20 ± 1.22^{a}
	Propria	1.00 ± 0.94^{a}	2.70 ± 0.48^{b}	2.20 ± 0.78^{c}	1.00 ± 0.94^{d}	1.90 ± 0.87^{bc}	1.20 ± 1.22^{acd}
	Muscularis	1.50 ± 0.84^{a}	2.50 ± 0.70^{c}	2.30 ± 0.94^{ac}	2.70 ± 0.67^{c}	1.50 ± 0.84^{ab}	1.00 ± 0.94^{b}
	Serosa	2.30 ± 0.48^{a}	3.00 ± 0.00^{b}	2.40 ± 0.51^{a}	2.40 ± 0.69^{a}	2.90 ± 0.31^{b}	1.40 ± 1.42^{a}

Note: Different superscripts in the same line show significant difference ($p < 0.05$). K1: Negative control (physiological NaCl injection), K2: Positive control (100 IU eCG, mated, 75 IU hCG), K3: 100 IU eCG, 75 IU hCG, K4: Artificial copulation, K5: 50 IU hCG and K6: 5 g GnRH.

According to Saruhan *et al*. [10], PR-A distribution in the oviduct of cattle was found to be more abundant during the follicular phase of estrous cycle, but intensity of this receptor is more strongly detected in early luteal phase. Aguilar and Reyley [33], reported that presence of PR-A in lamina epithelium of oviduct plays a role in providing various nutrients and growth factors for the oviduct tissue. Sagsoz *et al*. [20], stated that biological effects of progesterone will appear when progesterone interacts with its receptors, such as PR-A. Expression of PR-A in lamina epithelium, lamina propria and muscularis layer of rabbit oviduct was strongly expressed in K2, K3, K5 and K6 groups, meanwhile in groups K1 and K4, PR-A expression had moderate and weak intensities (**Table 4**). The difference in PR-A expression between groups of rabbits was due to differences in treatment, where K1 rabbits were not induced into pseudo-pregnancy but only injected with physiological NaCl, while K4 rabbits were induced by artificial copulation method which was thought to be inadequate at producing pseudo-pregnant rabbits compared to K2, K3, K5 and K6 groups.

Expression of immunoreactivity of PR-A in endometrium, uterine glands and myometrium of rabbit K1 - K6 are presented in **Table 5**. Absence of PR-A distribution in uterine endometrium of K1 rabbits was thought to be related to very low progesterone concentrations. Rabbits in K1 group were a group of rabbits that were not induced to become pseudo-pregnant. In uterine glands of K4 rabbits, PR-A expression was found in weak intensity. This is presumably because the uterine glands of K4 rabbits were not yet active in preparing endometrium for implantation. Rabbits in K4 group were induced by artificial copulation and showed different results from other groups of rabbits. According to Patel *et al*. [34], progesterone affects function of the uterus, as evidenced by finding of PR-A in lamina epithelial and stromal cells (lamina propria) of uterine endometrium. Wu *et al*. [35], explained that progesterone receptors in uterine tissue can be found in uterine glands which increase during luteal phase.

Table 5 Expression of PR-A in uterine tissue of K1 - K6 local rabbit.

Uterine layers	Treatment groups					
	K1	**K2**	**K3**	**K4**	**K5**	**K6**
Endometrium	1.00 ± 1.33^{a}	2.00 ± 0.67^{a}	2.60 ± 0.51^{b}	2.70 ± 0.67^{b}	2.60 ± 0.69^{b}	2.80 ± 0.42^{b}
Uterine Glands	0.60 ± 0.96^{a}	1.30 ± 0.94^{ac}	1.60 ± 1.07^{c}	0.80 ± 1.31^{ac}	1.20 ± 1.13^{ac}	3.00 ± 0.00^{b}
Myometrium	1.30 ± 1.15	2.70 ± 0.67	2.30 ± 0.94	2.70 ± 0.48	2.10 ± 0.99	3.00 ± 0.00
Perimetrium	1.50 ± 1.26^{ac}	2.10 ± 0.87^{c}	3.00 ± 0.00^{b}	3.00 ± 0.00^{b}	2.10 ± 0.99^{c}	0.90 ± 0.87^{a}

Note: Different superscripts in the same line show significant difference ($p < 0.05$). K1: Negative control (physiological NaCl injection), K2: Positive control (100 IU eCG, mated, 75 IU hCG), K3: 100 IU eCG, 75 IU hCG, K4: Artificial copulation, K5: 50 IU hCG and K6: 5 g GnRH.

Data on differences in distribution and expression of ERα and PR-A in oviduct and uterine tissue of rabbits obtained in this study indicates that hormones can be used as an induction method to produce pseudo-pregnant rabbits. However, these results need to be supported by data on the concentrations of estrogen and progesterone.

Conclusions

Method of using hormones to induce pseudo-pregnancy can be used to induce local rabbits to become pseudo-pregnant based on distribution of ERα and PR-A in oviduct and uterus.

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