

Morphological, Histological and Immunohistochemical Study of the Rabbit Uterus during Pseudopregnancy

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Abstract

Ten mature virgin rabbit does (4-5 months old) with mean weight of 2.4 kg were induced to ovulate; by intramuscular injection of HCG (50-70 IU). The day of induction was considered 0 days. Uteri were obtained from 14 h, 3, 7, 18 days post induction. At first stage of pseudopregnancy; the two uteri became hyperemic and slightly swollen. The uterine epithelium and endometrial glands epithelium were of columnar type with oval basal nuclei. The endometrial glands were few in number and small in size. The endometrial glands showed apocrine activity. The uterus had six longitudinal folds and the uterine epithelium form crypts. The endometrium had slight vascularization. At middle stage of pseudopregnancy, the two uteri became hyperemic and more swollen. This stage characterized by; mucosal folding, glandular formation, epithelial proliferation and thickening of the uterine wall. At last stage of pseudopregnancy; the two uteri became flaccid and more swollen. This stage characterized by dramatic changes in the uterine architecture in the form of: increased epithelial proliferation and crypt formation increase the complexity of luminal folding, increase in length, size and abundance of the uterine endometrial glands, increase in the uterine micro vascular development (increased abundance of the large microvessels and development of subepithelial capillary plexuses). Tunica vascularis could be demonstrated between the inner circular and outer longitudinal smooth muscle fibers of the myometrium at the all stages of pseudopregnancy. Telocytes with its characteristic morphology could be demonstrated in the myometrium of the rabbit uterus during pseudopregnancy. Cell-specific immuno-localization of progesterone receptors alpha (PRA) in the rabbit uterus during pseudopregnancy revealed that, there were mild, moderate and strong nuclear PRA immunostaining in the endometrial epithelial cells, endometrial glands and in the smooth muscles fibers of the myometrium of the first, middle and last stages of pseudopregnancy respectively.

The present study revealed that the morphological, histological and immunohistochemical changes in the rabbit uterus during pseudopregnancy resembled that occurred during early stage of pregnancy. These changes were characterized by dramatic changes in the uterine architecture in the form of: increased epithelial proliferation and crypt formation increase the complexity of luminal folding, increase in length, size and abundance of the uterine endometrial glands, increase in the uterine microvascular development. These uterine changes were accompanied with PRA immunolocalization in the uterine tissues.

Keywords: Rabbit; Uteri; Uterine glands; Pseudopregnancy; Progesterone receptor; Immunostaining

Introduction

The uterus is the major female reproductive organ of mammals, including humans. The female rabbit has a bicornuate duplex uterus. This type of uterus has two separate uteri. Each uterus has its own cervix, and the two cervixes open into a single vagina. Many of fertility problems in mammals are of uterine origin.

Pseudopregnancy is the appearance of signs of pregnancy as (maternal fur pulling, enlargement of mammary glands and milk production and nest building) in addition to histomorphological changes in uterine wall without the presence of an implanted embryo or fetus [1]. Pseudopregnancy lasts up to 18 days post ovulation and is related to the progestational influence of persistent corpora lutea [2,3]. Rabbits, unlike other mammals do not show regular estrus cycles, although a certain rhythm exists in their sexual receptivity. A doe may show no sexual receptivity in some cases as in molting, lactating or poorly nourished does because follicle development is suspended. Ovulation in rabbit is generally non-spontaneous or induced in nature, requiring the stimulus of mating for its induction. In the rabbit, ovulation occurs 10 to 14 h after copulation. Ovulation can also be induced by the sight of a sexually active male, by mating with a vasectomized or castrated (sterile) male, mouting by another female in an attempt to establish dominance, mechanical stimulation of the vagina, excessive handling by the owner or by hormones as exogenous

gonadotropins, electrical stimulation of the central nervous system [4] or stimulation of the central nervous system by drugs [5] and by stressful conditions as transportation [1].

One of the most encountering problems in rabbit breeding is the pseudopregnancy or false pregnancy. Once a doe reaches sexual maturity, she may go through periodic false pregnancies. Pseudopregnancy may lead to many disorders in rabbits as mild alopecia, uterine disorders, mastitis, bite wounds and abscesses [1]. The pseudopregnant rabbit is a model commonly used to study reproductive endocrinology [6]. Induction of a local pseudopregnancy via levonorgestrel-loaded microspheres was used for the treatment of endometriosis in a rabbit model with fewer side effects [7,8]. There was little information

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describing the rabbit uterus during pseudopregnancy so, the aim of the present study is to give more information in the histomorphology and progesterone receptors alpha immunohistochemistry of the rabbit uterus during pseudopregnancy.

Materials and Methods

Source of the animals

Female New Zealand white rabbits were obtained from animal house, faculty of medicine, Assiut University.

Experimental design

Mature rabbit does (4-5 months old) with mean weight of 2.4 kg were putted under controlled and stable conditions of light (day light and 7 h. electric light), temperature (22-25°C), ventilation and humidity. The does were induced to ovulate by intramuscular injection of HCG (50-70 IU Choriomon, IBSA Institut Biochimique S.A, Lugano, Switzerland). The day of induction was considered 0 days. Uteri were obtained from 14 and 40 h, 3, 7, 18 days post induction of ovulation.

Histological preparation

Paraffin sections stained with haematoxylin and eosin stain for general histological examination: Uteri were dissected as soon as possible after slaughtering and were immediately fixed with 10% neutral buffered formalin or Bouin's fluid. The fixed materials were dehydrated in ascending grades of ethanol, cleared in methyl benzoate and then embedded in paraffin wax. Cross serial paraffin sections at 5 µm thicknesses were cut and stained with haematoxylin and eosin stain for general histology examination [9].

Immunohistochemical detection of progesterone receptors alpha (PRA or PRα) in paraffin sections: by using Progesterone Receptor (Clone SP2) Rabbit Monoconal Antibody Cat.#RM-9102-S0 and Ultravision Detection System (Anti-Polyvalent, HRP/DAB), Thermo Fisher Scientific, USA.

1. The fixed materials were dehydrated in ascending grades of ethanol, cleared in methyl benzoate and then embedded in paraffin wax.

2. Sections (3-5 µm) of paraffin-embedded tissue were dewaxed by immersion of slides in xylene for 3 × 5 min, rehydrated slides in 100% I, 100%II, 95%, 80% ethanol for 3 min each, and rinsed in PBS pH 7.4 (3 × 5 min).

3. Endogenous peroxidase was inhibited by 3% hydrogen peroxide for 10 min at room temperature followed by washing in PBS pH 7.4 (4 × 5 min). (3% hydrogen peroxide prepared by adding 3 mL of hydrogen peroxidase block to 97 mL distilled water).

4. For Antigen retrieval, the slides were placed in 10 mM sodium citrate buffer (pH 6.0) and heat samples near boiling (95-98) in water bath for 20 min followed by cooling for 20 min at room temperature.

5. Sections were then rinsed in PBS pH 7.4 (3 × 1 min).

6. Sections were covered with Ultra V block (components were shown in table 1 Part II, Thermo Fisher Scientific, USA) for 5 min at room temperature to block non-specific background staining. Note: the blocking time didn't exceed 10 min.

7. Sections were then incubated with the primary antibodies for 1 h at room temperature or overnight according to the data sheet of antibody.

8. The slides were washed with PBS pH 7.4 (4 × 5 min), followed by incubation with a biotinylated secondary antibody by adding drops on the section for 10 min at room temperature.

9. The slides were thereafter rinsed in PBS (pH 7.4, 3 × 5 min) followed by incubation with drops of streptavidin- peroxidase complex (Thermo Fisher Scientific, USA) for 10 min at room temperature. The slides were thereafter rinsed in PBS pH 7.4, (4 × 5 min).

10. Visualization of the bounded antibodies was carried out with add 1 drop of (DAB (diaminobenzidine) Plus chromogen to 2 mL of DAB Plus substrate, mix by swirling and apply drop on the tissue, Incubate for 5-10 min at room temperature.

Note: All incubations were performed in a humid chamber to avoid drying of the tissue (A humidified chamber is a container with a wet paper towel).

The sections were counterstained in Harris haematoxylin for 30 seconds.

11. The sections were dehydrated in a graded series of alcohols (ethanol 95%, ethanol, 100% I and II), cleared with xylene, and mounted with DPX. Negative controls were performed by omission of the primary antibodies.

Immunohistochemical staining were examined by OLYMPUS BX51 microscope and the photos were taken by OLYMPUS DP72 camera adapted into the microscope

Ethical Statement

All experiments were carried out in accordance with the Egyptian laws and University guidelines for the care of experimental animals. All procedures of the current study have been approved by the Committee of Faculty of veterinary medicine, Assiut University, Egypt.

Results

The morphological study of the rabbit uterus during pseudopregnancy revealed that at 14h post injection of an ovulatory dose of human chorionic gonadotropin (HCG); the two uteri became slightly hyperemic and slightly swollen. At 3days of pseudopregnancy; the two uteri became hyperemic and swollen. At 7- 18 days of pseudopregnancy; the two uteri became flaccid and more swollen (Figure 1). The histological study of the rabbit uterus during pseudopregnancy revealed that the uterine wall was composed of three layers. From the uterine lumen outward they were as follows: The endometrium; the mucosa, the myometrium, the thick muscular layer and the perimetrium; the serosa. The endometrium was consisted of lamina epithelialis of simple columnar type and connective tissue lamina propria containing leucocytic infiltration and endometrial glands. The myometrium was formed of an inner circular (IC) and outer longitudinal (OL) smooth muscle fibers separated by tunica vascularize (layer of loose connective tissue containing blood vessels). The perimetrium was formed of a mesothelium and submesothelial loose connective tissue. The present study revealed that, the uterus at 14 h-3 days had a large lumen and thin wall when compared to 3-7 days which had more thicker wall and narrower lumen and 7-18 days which had the thickest wall and the narrowest lumen (Figure 2).

At first stage of pseudopregnancy; 14 h-3 days post injection of an ovulatory dose of HCG, the uterine epithelium and endometrial glands epithelium were formed of columnar epithelium with oval basal nuclei. The endometrial glands were few in number and small in size. The

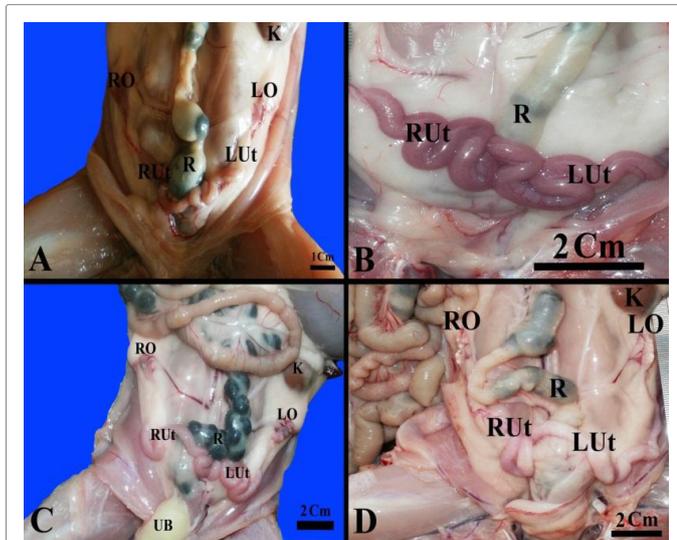


Figure 1: Photograph of female rabbit A; 14 h, B; 3 days, C; 7 days and D; 18 days post injection of an ovulatory dose of HCG showing right uterus (Rut), left uterus (LUt), right ovary (RO), left ovary (LO), left kidney (K), rectum (R) and urinary bladder (UB). Note in A: the two uteri became slightly hyperemic and slightly swollen in B: The two uteri became hyperemic and swollen and in (C&D) the two uteri became flaccid and more swollen.

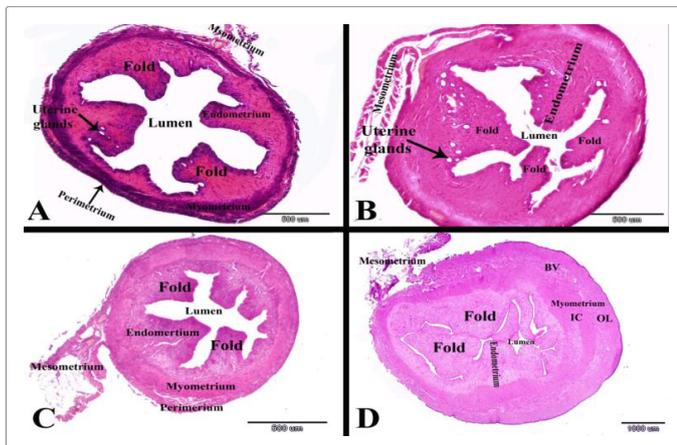


Figure 2: Photomicrograph of cross section of rabbit uterus at different stages of pseudopregnancy; A; 14 h, B; 3 days, C; 7 days and D; 18 days post injection of an ovulatory dose of HCG showing, endometrium, myometrium [inner circular (IC) and outer longitudinal (OL) smooth muscle fibers separated by tunica vascularize (containing blood vessels)] and perimetrium of the uterine wall, uterine folds, uterine glands and mesometrium. Note that the uterus at 14 h-3 days had a large lumen and thin wall when compared to 3-7 days which had more thicker wall and narrower lumen and 7-18 days which had the thickest wall and the narrowest lumen. Original magnification, (A, B&C): 40X, scale bar=500 μm and d: 25X, scale bar=1000, Hematoxylin and Eosin stain.

endometrial glands were lined by columnar cells with oval basal nuclei and were surrounded by myoepithelial cells. The endometrial glands were contained secretion formed by apocrine activity. The uterus had six longitudinal folds and the uterine epithelium form crypts. The endometrium at this stage had slight vascularization (Figure 3). The middle stage of pseudopregnancy (3-7 days post injection of an ovulatory dose of HCG) was characterized by; mucosal folding, glandular formation, epithelial proliferation and thickening of the uterine wall (Figures 4 and 5). The last stage of pseudopregnancy (7-18 days post injection of an ovulatory dose of HCG) was characterized by dramatic changes in the uterine architecture in the form of: increased

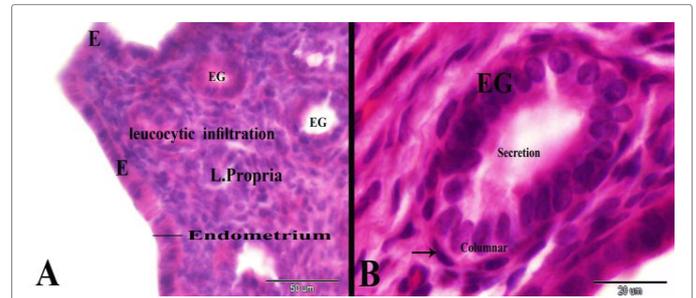


Figure 3: Photomicrograph of the endometrium of rabbit uterus at 14 h-3 days of pseudopregnancy; A: showing the endometrium; consisted of lamina epithelialis of simple columnar type (E) and connective tissue lamina propria containing leucocytic infiltration and few small endometrial glands (EG). B: showing the endometrial gland lined by columnar cells with oval basal nuclei and surrounded by myoepithelial cells (arrow). The endometrial gland contained secretion formed by apocrine activity. Original magnification, A: 400X, scale bar=50 μm and B: 1000X, scale bar=20, Hematoxylin and Eosin stain.

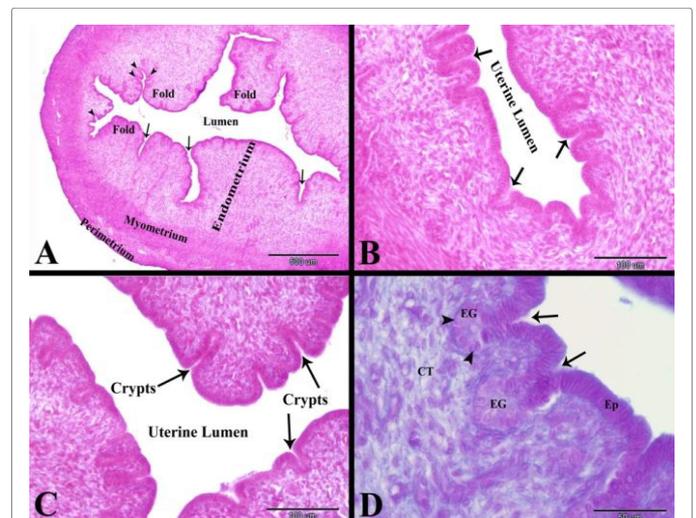


Figure 4: Photomicrograph of the rabbit uterus at 3-7 days of pseudopregnancy; A: Showing endometrium, myometrium, perimetrium, uterine lumen, endometrial folding (arrow) and glandular formation (arrow head). B and C: Showing crypt formation (arrow). D: Showing epithelial invaginations (crypts) and proliferation (arrow) which formed the endometrial glands (EG), lamina epithelialis formed of simple columnar type (Ep) and connective tissue lamina propria (CT). Original magnification, A: 40X, scale bar=500 μm, B&C: 200X, scale bar=100 and D: 400X, scale bar=50 μm, Hematoxylin and Eosin stain.

epithelial proliferation and crypt formation increase the complexity of luminal folding, increase in length, size and abundance of the uterine endometrial glands. The uterine gland or endometrial gland was a simple tubular gland formed by invagination of the uterine endometrium. This stage was also characterized by an increase in the uterine microvascular development in the form of increased abundance of the large microvessels and development of subepithelial capillary plexuses (Figure 6). Telocytes with its characteristic morphology could be demonstrated in the myometrium of the rabbit uterus during pseudopregnancy (Figure 7).

Cell-specific immuno-localization of progesterone receptors alpha (PRA) in the rabbit uterus during pseudopregnancy revealed that, at 14 h post induction of pseudopregnancy there were slight nuclear PRA immunostaining in the endometrial epithelial cells, endometrial glands and inner circular and outer longitudinal smooth muscles fibers of

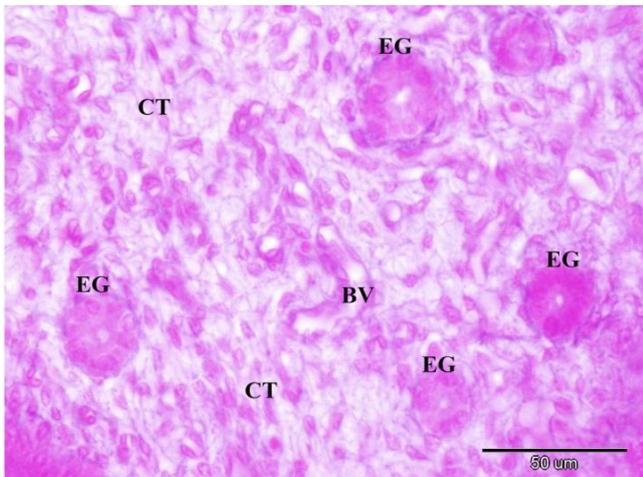


Figure 5: Photomicrograph of the endometrium of rabbit uterus at 3- 7 days of pseudopregnancy showing, connective tissue lamina propria (CT)containing blood vessels (BV) and endometrial glands (EG). Original magnification, 400X, scale bar=50 µm, Hematoxylin and Eosin stain.

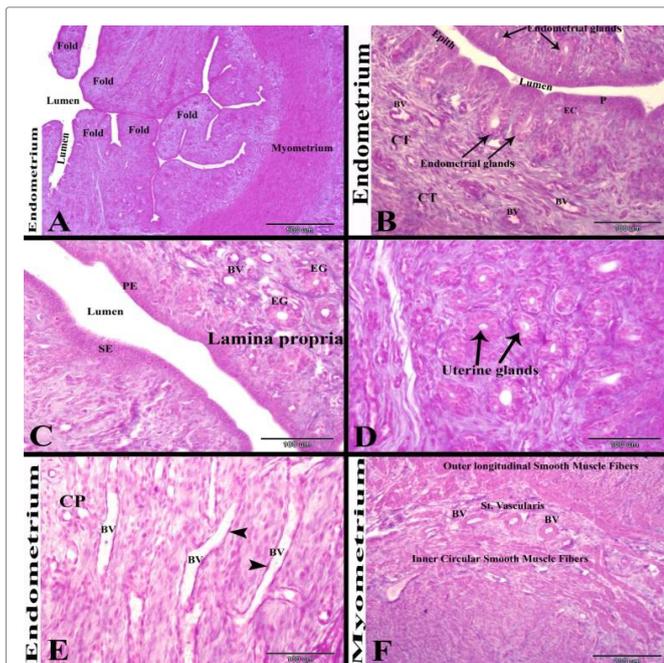


Figure 6: Photomicrograph of the rabbit uterus at 18 days of pseudopregnancy. A: Showing narrow lumen, highly folded endometrium and myometrium. B: Showing narrow uterine lumen, endometrial epithelium (Epith) which proliferated in some area (P) to form epithelial cords(EC)and connective tissue lamina propria (CT) containing blood vessels (BV) and endometrial glands (EG). C: showing uterine lumen, pseudostratified (PE) and stratified endometrial epithelium, connective tissue lamina propria containing blood vessels (BV) and endometrial glands (EG). D: showing abundant uterine glands. E: showing increased abundance of the large microvessels (BV) and development of subepithelial capillary plexuses (CP). Note the endothelial lining of these blood vessels (arrow head). F: showing stratum vascularis (St vascularis); appeared as loose connective tissue containing numerous blood vessels (BV) between the inner circular and outer longitudinal smooth muscle fibers of the myometrium. Original magnification, A: 40X, scale bar=500 µm, (B-E): 200X, scale bar=100 and F: 100X, scale bar=200 µm, Hematoxylin and Eosin stain.

the myometrium. At 3 days of pseudopregnancy there were moderate nuclear PRA immunostaining in the endometrial epithelial cells,

endometrial glands and inner circular and outer longitudinal smooth muscles fibers of the myometrium. At 7-18 days of pseudpregnancy there were strong nuclear PRA immunostaining in the endometrial epithelial cells, stroma cells, endometrial glands and in the smooth muscles fibers of the myometrium (Figure 8).

Discussion

The present study revealed that the morphological, histological and immunohistochemical changes in the rabbit uterus during pseudopregnancy resembled that occurred during early stage of pregnancy. Herein, the uterus at 14 h-3 days had a large lumen and thin wall when compared to 3-7 days which had more thicker wall

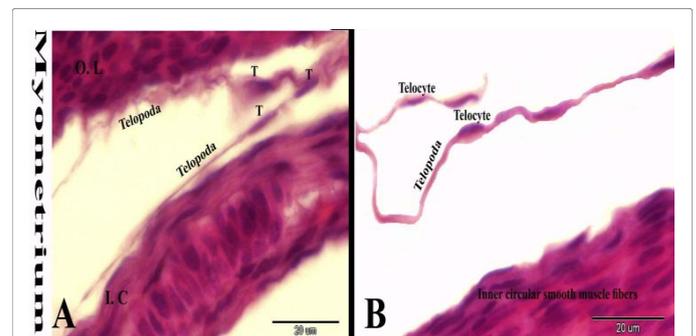


Figure 7: Photomicrograph of the rabbit uterus during pseudopregnancy showing, telocytes (T) with its characteristic telopoda in the myometrium between the inner circular and outer longitudinal smooth muscle fibers of the myometrium. Original magnification, A & B: 1000X, scale bar=20 µm, Hematoxylin and Eosin stain.

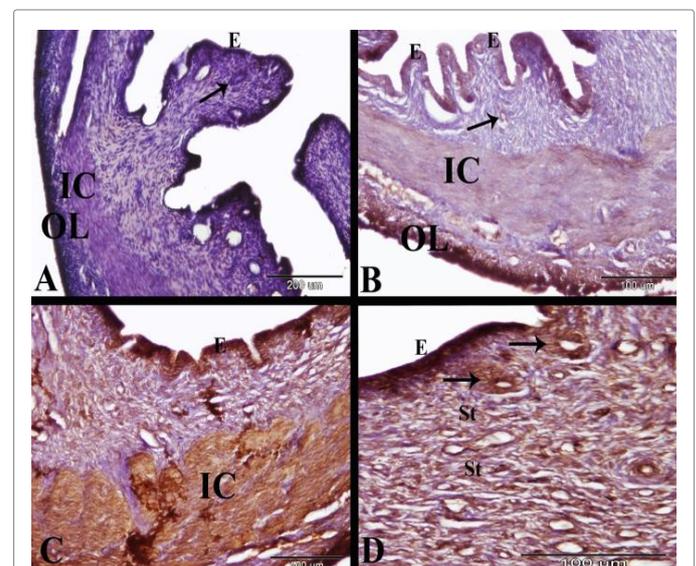


Figure 8: Photomicrograph showing cell-specific immuno-localization of progesterone receptors alpha (PRA) in the rabbit uterus during pseudopregnancy. A: 14 h. post induction of pseud pregnancy showing slight nuclear PRA immunostaining in the endometrial epithelial cells (E), endometrial glands (arrow) and inner circular (IC) and outer longitudinal (OL) smooth muscles fibers of the myometrium. B: 3 days of pseudpregnancy showing moderate nuclear PRA immunostaining in the endometrial epithelial cells (E), endometrial glands (arrow) and inner circular (IC) and outer longitudinal (OL) smooth muscles fibers of the myometrium. C&D: 7-18 days of pseudpregnancy showing strong nuclear PRA immunostaining in the endometrial epithelial cells (E), stroma cells (St), endometrial glands (arrow) and in the smooth muscles fibers of the myometrium (IC). Original magnification, A: 100X , scale bar=200 µm, (B, C and D) 200X, scale bar=100 µm.

and narrower lumen and 7-18 days which had the thickest wall and the narrowest lumen. The uterine wall thickness increased during pregnancy [10]. This increase in thickness was due to endometrial surface epithelium and gland proliferation and hypertrophy, increased myometrial extracellular matrix and myometrial smooth muscle hypertrophy [11]. Female rabbit have two separate uteri and the changes of pregnancy or pseudopregnancy occurs in the two uteri but in some mammalian species as the tammar wallaby (*Macropus eugenii*) these changes occurs in one uterus [12].

At first stage of pseudopregnancy (14 h-3 days post injection of an ovulatory dose of HCG), the two uteri became hyperemic and slightly swollen. The uterine epithelium and endometrial glands epithelium were of columnar type with oval basal nuclei. No ciliated cells could be demonstrated in rabbit uterus during pseudopregnancy [13]. Ciliated cells predominated at oestrus and day 21 of pseudo pregnancy in rabbit but were sparse at other times [14]. The endometrial glands were few in number and small in size. The endometrial glands showed apocrine activity. Endometrial secretion appears much sooner during the HCG-induced pseudopregnancy than in a normal pregnancy and maximal secretory capacity is reached after 7 days of the pseudopregnancy [3].

At middle stage of pseudopregnancy (3-7 days post injection of an ovulatory dose of HCG), the two uteri became hyperemic and more swollen. This stage characterized by; mucosal folding, glandular formation (endometrial adenogenesis), epithelial proliferation and thickening of the uterine wall [13]. Similar changes were occurred in pseudopregnant ferrets [15] and pregnant marsupials [16]. These changes were due to increased plasma progesterone level [17]. In some animals the early endometrial changes play an important role in maintenance of the viable blastocyst and inhibition of further conceptus growth during diapause [12]. Uterine histological changes are in relation with the differentiation of the embryo [18].

At last stage of pseudopregnancy (7-18 days post injection of an ovulatory dose of HCG), the two uteri became flaccid and more swollen. This stage characterized by dramatic changes in the uterine architecture. The normal pre implantation endometrial morphology developed in rabbit after 7 days of hCG injection [2]. These changes were increased epithelial proliferation and crypt formation, increased the complexity of luminal folding, increased in length, size and abundance of the uterine endometrial glands [13,19]. The number and the size of uterine gland increased during the pregnancy period of the rat and this was controlled by progesterone [20]. The importance of endometrial surface epithelium and of gland hyperplasia and hypertrophy for the conceptus implantation and nourishment [11]. Implantation is a critical proses for placentation [21]. In addition, increased in the uterine microvascular development (increased abundance of the large microvessels and development of subepithelial capillary plexuses) could also be observed. Tunica vascularis could be demonstrated between the inner circular and outer longitudinal smooth muscle fibers of the myometrium at the all stages of pseudopregnancy. The number and the size of blood vessels changed during the pregnancy period of the rat and this was controlled by progesterone [20]. Endometrial vascularity subepithelial capillary plexus increased in pseudopregnant rabbit than in the estrous rabbit [22].

The present study revealed that, there were mild, moderate and strong nuclear PRA immunostaining in the endometrial epithelial cells, stromal cells, endometrial gland cells and in the smooth muscles fibers of the myometrium of the first, middle and last stages of pseudopregnancy respectively. Progesterone is the principal hormone of pregnancy. It is required in all mammals for maintains the uterus in

a quiescent state and for maternal support of conceptus (embryo/fetus and associated membranes) survival and development [20,22]. The physiological effects of progesterone are mediated by interaction of the hormone with specific intracellular progesterone receptors (PRs) that are expressed from a single gene as two protein isoforms; progesterone receptors alpha (PR-A) and progesterone receptors beta (PR-B) and that are members of the nuclear receptor superfamily (NRS) of transcription factors [23,24]. Several *in vivo* and *in vitro* studies have demonstrated that the PR-A and PR-B proteins have different transcription activation properties when liganded to progesterone [25-32]. These studies elucidated that the ablation of PR-A does not affect response of the mammary gland or thymus to progesterone but results in severe abnormalities in ovarian and uterine function leading to female infertility. Whereas the ablation of PR-B does not affect ovarian, uterine or thymic responses to progesterone but reduce mammary ductal morphogenesis and alveologenesis during pregnancy. Thus PR-A in uterus are necessary for female fertility.

PR immunoreactivity in uterine epithelium of pseudopregnant rabbits was low during the first five days of pseudopregnancy and became greater on day 7 [33]. The number of cytosolic and nuclear progesterone receptors increased at the end of pseudopregnancy (between days 13 and 18) in rabbit (Quirk and Currie, 1984). Uterus of human and mouse expressed nuclear PR in epithelial, stromal and muscle cells [34]. Progesterone and by default its receptors (PRA) maintains the uterus in a quiescent state and appears to be the principal hormone required for maintenance of a conceptus supportive environment in all species [20]. The progesterone receptor regulates implantation, decidualization, and glandular development via a complex paracrine signaling network [35]. Progesterone and progesterone receptor (PR) regulate endometrial gland morphogenesis and terminal differentiated function to maintain pregnancy [23] by regulation of a number of known gland-specific genes [36].

Interactions of progesterone with its PRA in the endometrial epithelial cells regulate implantation-related genes in the luminal endometrial epithelial cells and govern implantation, conceptus survival and growth [37]. One of the most important changes in the endometrial epithelial cells was the plasma membrane transformation [21] and this may be depending upon PRA in endometrial epithelial cells. It is hypothesized that progesterone act in the PR-positive endometrial luminal and glandular epithelia to indirectly stimulate trophoblast proliferation and production of interferon (IFN) tau. IFN tau, the pregnancy recognition hormone abrogates the release of luteolytic pulses of prostaglandin F2 alpha (PGF) from the endometrial epithelium by indirect way [22,38]. PR-positive stromal cells produce several growth factors, such as hepatocyte growth factor (HGF) and fibroblast growth factors-7 and-10 (FGF-7, FGF-10). These factors have receptors expressed specifically in the endometrial epithelia and so they mediate epithelial-mesenchymal interactions that are crucial for support of pregnancy [38].

Endometrial glands were present in all mammalian uteri [39]. In humans and animals like; rodents and sheep, secretory products of the endometrial glands were necessary for peri-implantation conceptus survival and development in addition to establishment of uterine receptivity and blastocyst implantation [36,38,40,41]. Uterine glands secrete bioactive substances that regulate uterine luminal fluid (ULF) homeostasis and regulate uterine receptivity for blastocyst implantation. These substances regulate uterine receptivity genes and gland-specific genes [36]. During implantation placental lactogen (PL) stimulates endometrial glandular epithelial cells proliferation

and production of secretory proteins, such as uterine milk proteins (UTMP) and osteopontin (OPN) [38]. Uterine glands and their secretory products were critical regulators of conceptus growth and development during the first trimester [41]. During mid-pregnancy, some placental cells produce growth hormone GH that act on a progesterinized uterus to stimulate endometrial glandular epithelial cells hyperplasia, hypertrophy and maximal secretory function [38]. In the rabbit, sheep, and pig, regulation of endometrial gland development and differentiated function during pregnancy involved sequential actions of ovarian steroid hormones, pregnancy recognition signals, and lactogenic hormones from the pituitary or placenta.

Telocytes with its characteristic morphology could be demonstrated in the myometrium of the rabbit uterus during pseudopregnancy. Telocytes (TCs) are a distinct type of interstitial cells characterized by a small cell body and extremely long and thin telopodes (Tps). The presence of TCs has been documented in many tissues and organs. TCs may function in repair and regeneration of different tissues and organs, including heart, lung, skeletal muscle, skin, meninges and choroid plexus, eye, liver, uterus and urinary system [42,43]. Telocytes were identified in human fallopian tube and uterus and they express estrogen and progesterone receptors [44]. TCs were present in human non-pregnant and pregnant myometrium as a distinct cell type; c-kit (CD 117) and CD34 positive cells. TCs seem to have no excitable properties similar to smooth muscle cells [45].

The presence of active CL and by default its progesterone secretion was the main factor responsible for all uterine changes which occurred during pseudopregnancy in rabbits. Tissue and serum progesterone levels increased significantly during pseudopregnancy in rabbit [6]. The uterine changes were lasted until the regression of CL at 18 day post injection of an ovulatory dose of HCG [17] or about half the time of gestation [4]. The presence of the conceptus appears to be not necessary for maintenance of the CL in rabbits until maternal recognition of pregnancy. Maternal recognition of pregnancy in rabbits occurs between Days 10-18 post coitum and that the conceptus provides a signal to maintain continued corpus luteum progesterone secretion [46,47]. The rabbit conceptus can be decrease or inhibit the ovarian responsiveness to PGF-2 α and prolongs the life span of the CL by production of luteotrophic and/or antiluteolytic factors. So in pseudopregnancy the CL is functional for only 16-18 days and it is then that the CL of pseudopregnant rabbits begins to regress as a result of increased luteal responsiveness to PGF-2 α .

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