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Microscopic Evaluation of Uterine Mucosa in Pregnant and Non-pregnant Rats

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Abstract: Pregnancy is associated with dramatic changes in morphological structure of endometrium and leucocytes infiltration within the uterus. The main aim of this study was to evaluate compare the histological structure of uterine mucosal of pregnant and non-pregnant rats. A total of eight healthy Spraque-dawley rats were used in this study. They were divided into two groups; pregnant and non-pregnant groups with 4 rats per group. For the pregnant group, the animals were euthanized at 14 days of gestation period. Uterine samples were taken and processed for histological examination using H&E staining and PAS stain for secretory cell count. Histological structure of the uterine mucosal layer of pregnant and non-pregnant rats was significantly difference. Infiltration of PMN cells was higher in non-pregnant rats than those of pregnant rats, whereas, infiltration of lymphocytes and macrophages were diffusely scattered in pregnant rather than the non-pregnant rats.

Key words: Uterine mucosa, Pregnant, Non-pregnant, Leucocytes, Rat

Introduction

Uterine mucosa or also known as endometrium consists of epithelium, endometrial stroma or lamina propria and uterine glands. The epithelium of the uterine mucosa is lining with a simple columnar that composed of mixture of ciliated cells and non-ciliated cells. Non-ciliated cells also known as secretory cells. The ciliated cells functions in transportation of ovum whereas non-ciliated cells or secretory cells secrete granulated materials that associated with the nutrition and protection of ovum and embryo. The ratio of ciliated cells to secretory cells is influenced by the hormones. The secretory cells were also observed more frequently than ciliated cells toward posterior end of oviduct (Johnson and Foley, 1972).

In general, lamina propria or endometrium stroma consists of a network of collagen reticular fibers with numerous fibroblast-like cells, the fusiform cells. The area is cellular richly vascularized connective tissue and is directly adjacent to the myometrium. Invaginations of uterine mucosa for many times result in formation of many simple tubular or branched tubular glands called uterine gland. The epithelium of these glands is similar to that of uterine epithelium but the non-ciliated cells are rarely present. The function of these glands is to secrete the material that is rich in glycogen that provides nutrition for fertilized ovum (Young and Heath, 2000).

Leucocytes were infiltrated into the endometrium in normal and healthy animal. It is important as host mucosal defense mechanism against infection and the phagocytic activity is similar to those leucocytes of peripheral blood (Anderson *et al*., 1985). The leucocytes can be divided into two categories based on their action which are non-specific and specific. The non-specific immune leucocytes consist of polymorphonuclear cells (neutrophil, basophil and eosinophil) and macrophages or monocytes. It involves in a process of chemotaxis, adherence, ingestion and digestion and these types of leucocytes are effective without prior exposure to a specific pathogen. A specific leucocytes cell involves the lymphocytes cells and the responses are highly specific for a particular pathogen. The reproductive failure due to disorder of implantation or because of infection during pregnancy is still a major problem to the farmer or breeder.

The present study was conducted with the main aim to evaluate the histological structure of uterine mucosal layer of pregnant and non-pregnant rats. In addition, the number of leucocytes infiltration into the endometrium of pregnant and non-pregnant rats was also determined.

Materials and Methods

Experimental Animals: A total of eight healthy Spraque-dawley rats were used in this study. The rats were supplied by the Animal Breeding Unit, Faculty of Veterinary Medicine, Universiti Putra Malaysia. The rats were divided into two groups; pregnant and non-pregnant groups with four rats per group. All the rats were euthanized by giving overdose of Ketamine and Xylazine. For the pregnant group, the rats were euthanized at 14 days of gestation period. The rats were given commercial pellet and water *ad libitum* throughout the study. The protocol was approved by the Faculty's Ethics Committee.

Histological Examination: Upon euthanasation, the whole portion of uterus was removed and fixed in 10% formalin. After a fixation period of 48 hr, the uteri of both pregnant and non-pregnant rats were cut at five different parts:

the After a fixation period of 48 hr, the uteri of both pregnant and non-pregnant rats were cut at five different parts: the fallopian tube, proximal uterine horn, middle uterine horn, distal uterine horn and uterine body. The tissues were trimmed into 0.5 cm slices and fixed in 10% buffered formalin for 48 hr before being processed for histological examination. The tissues were sectioned at 5 µm thick and stained with Haematoxylin and Eosin and Periodic Acid Schiff (PAS) for secretory cell count. The slides were examined under light microscope for the presence of leucocytes in the endometrium.

Analysis: For each section of the uterus, the numbers of positive-staining cells were examined to estimate the size of leucocytes and goblet cells in the endometrium. The presence of these cells were scored as absent (+), occasional (++), moderate (+++), high (++++) and profuse (+++++) according to the method described by Zamri-Saad (1987).

Results and Discussion

Histological Examination: In the non-pregnant rats, the epithelium of endometrium consist only a single layer. The epithelium cells were in the form of tall columnar. The secretory cells were less numerous and appeared slightly increased in size and formed balloon-like bulges on the luminal surface. The epithelium of uterine gland consists of a single layer of columnar epithelial cells. The lamina propria of non-pregnant rats was thicker.

In the pregnant rats, the epithelium of the uterine mucosal layer had increased in numbers and layers (Fig. 1). The secretory cells were numerous and hypertrophied especially in the body of uterus. These cells were stained pink after counter stain with PAS (Fig. 2). The uterine glands were tortuous and the epithelium of these glands was thickened. The lamina propria of pregnant rats was decreased in thickness.

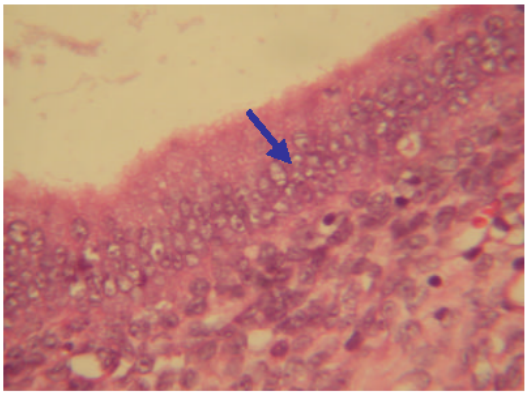

Fig. 1: Histological examination of the horn of pregnant uterus shows the epithelium of uterine mucosal layer increase in thickness (arrow). (H&E x40)

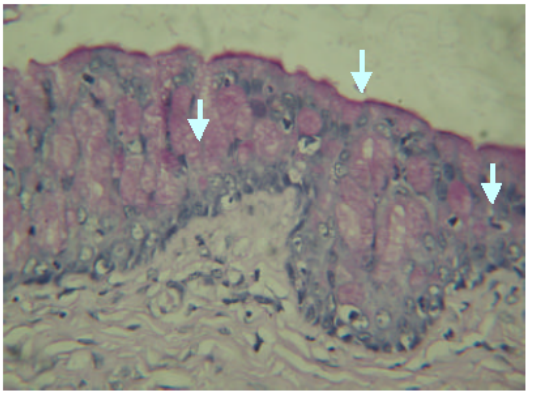

Fig. 2: Histological examination of the body of pregnant uterus shows the present of numerous secretory cells (arrows). Note that the cells were hyperplasia and hypertrophy. (PAS x40)

The Occurrence of Intrauterine Leucocytes and Secretory Cells in Pregnant and Non-pregnant Rats

Polymorphonuclear (PMN) Cells in the Epithelium: The results of the occurrence of polymorphonuclear (PMN) cells infiltration in epithelium of uterine mucosa in all portion of uterus is showed in Fig. 3. In general, the number of PMN in the epithelium of endometrium was higher in the non-pregnant uterus as compared to the pregnant uterus. In non-pregnant rats, the average score for all rats was +++ or moderate in fallopian tube (FP), proximal uterine horn (Pro.UH), distal uterine horn (Dis.UH) and body of uterus (Body). However, in the middle of uterine horn (Mid.UH), the average score was ++ or occasional. In pregnant rats, the infiltration of PMN cells was much lower than that of the non-pregnant rats. The PMN cells were not found (score + or absent) in the FP, Pro.UH and Dis.UH and body of uterus of the pregnant rats, whereas average score of ++ (occasional) was observed at the Mid.UH.

Polymorphonuclear (PMN) Cells in the Lamina Propria: Fig. 4 showed the average score of polymorphonuclear (PMN) cells in the lamina propria of pregnant and non-pregnant rats. In comparison with the infiltration of PMN cells in the epithelium, the occurrence of PMN cells in the lamina propria was higher than in the epithelium for both the pregnant and non-pregnant rats. Generally, the number of PMN in lamina propria of non-pregnant uterus was higher than that in the pregnant uterus. Non-pregnant rats have average score of ++++ (high) at the FP, Mid.UH, Dis.UH and

body of the uterus and score of +++++ (profuse) was found at the Pro.UH. In the pregnant rats, average score was ++ (occasional) at the FP, Pro.UH and Dis.UH, whereas at the region of Mid.UH and body of uterus, the score was +++ (moderate).

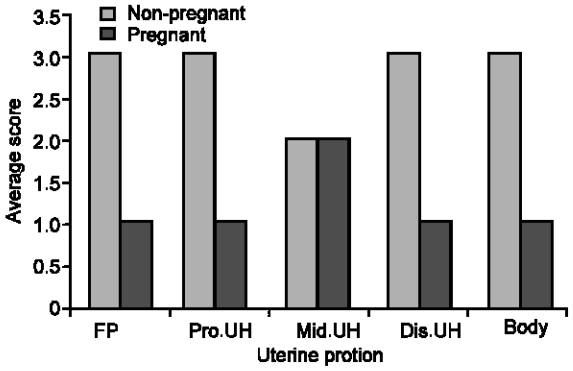

Fig. 3: The occurrence of polymorphonuclear cells in the epithelium of uterine mucosa

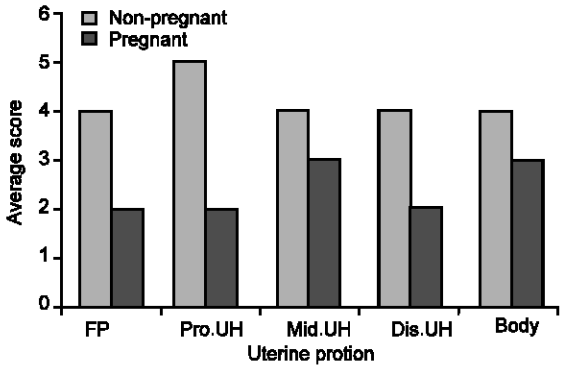

Fig. 4: The occurrence of polymorphonuclear cells in the lamina propria of uterine mucosa of the pregnant and non-pregnant rats

Lymphocytes and Macrophages in the Endometrium: Figure 5 revealed the average score of infiltration of lymphocytes and macrophages in the endometrium. The results showed that pregnant rats have higher number of infiltration of both cells in the endometrium. In non-pregnant rats, the average score was +++ (moderate) at the FP, Mid.UH and Dis.UH, whereas the pregnant rats have score of ++++ (high) at the same portion. The Pro.UH of non-pregnant rats have average score ++ (occasional) and pregnant rats have average score +++ (moderate). The body of uterus of both non-pregnant and pregnant rats have higher average score as compared to other portion, whereby the non-pregnant rats have score of ++++ (high) and the pregnant have average score of +++++ (profuse).

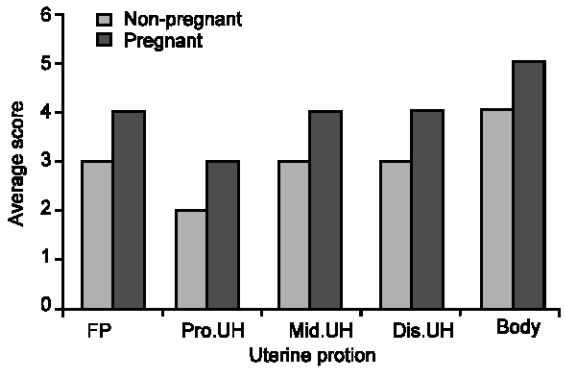

Fig. 5: The occurrence of lymphocytes and macrophages in endometrium of the pregnant and non-pregnant rats.

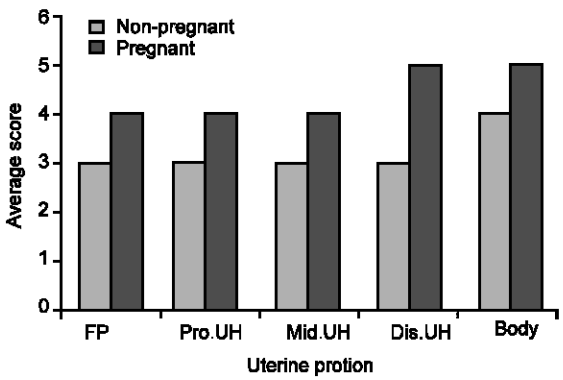

Fig. 6: The occurrence of secretory cells at the epithelium of uterine mucosa of the pregnant and non-pregnant rats.

Secretory Cells in the Epithelium of Uterine Mucosa: The occurrence of secretory cells at the epithelium of uterine mucosa is summarized in Fig. 6. The results showed that pregnant rats have more number of secretory cells as compared to the non-pregnant rats. In the non-pregnant rats, the average score was +++ (moderate) at four portions which were the FP, Pro.UH, Mid.UH and Dis.UH and the number had increased at the body of uterus with average score of ++++ (high). In the pregnant uterus, the average score was ++++ (high) at the portions of FP, Pro.UH and Mid.UH. However, the occurrence of secretory cell was profuse (+++++) at the Dis.UH and body of uterus in the pregnant rats.

The uterine mucosa or endometrium undergoes dynamic changes in preparation for implantation and the changes involve the morphology of the endometrium and also the occurrence of leucocytes infiltration into the endometrium.

The concepts of these changes are poorly understood. However, according to Fazleabas and Strakova (2001), they suggested that the change of morphological structure of endometrium is to ensure prolonged maintenance of endometrial function during gestation and facilitate trophoblast invasion. Environmentally modifications of leucocytes also have major pregnancy-associated functions that include facilitation of implantation, modulation of maternal uterine vasculature, supply of growth factors to the placenta, promotion of trophoblast differentiation and facilitation of parturition (Hunt *et al*., 2000).

In this study, the morphological changes of endometrium was observed based on the epithelium lining of the mucosa, the thickness of the lamina propria, the numbers of secretory cells and the appearance of uterine glands. There was a significantly different of the morphology of endometrium between pregnant and non-pregnant rats. Microscopic examination of the uterine mucosa of non-pregnant rats showed that the epithelium was lining with a single layer of simple columnar in almost all five portions of uterus. These findings agree with the results of Lauschova (1999) that has demonstrated decreased in the epithelium mitotic Fig.s of estrogenized animals. However, the epithelium will increase in numbers during estrus or proliferative phase in which the uterus starts producing progesterone hormone as a normal cycle as shown in Fig. 1 (Young and Heath, 2000). Thus, this fact support to the finding of this study in pregnant rats that also has increased in number and height of cell lining the epithelium of uterine mucosa because progesterone is important hormone for maintaining pregnancy. In bovine oviducts, Uhrin (1992) found that the highest values of the epithelium height occur during estrus and decreased during diestrus. Similar findings were also reported by other authors (Tarara *et al*., 1987; Enders, 1991 and Enders, 1997). They found that the response to pregnancy is characterized by hypertrophy of the surface epithelium.

To our knowledge, there is limited study has been done on secretory cell of the endometrium. In this study only the numbers and appearance of the secretory cells were described. The secretory cells in non-pregnant rats were found among predominated ciliated cells at all five portions. Only one of the non-pregnant rats demonstrated the occurrence of secretory cells that was tall and bulging like balloon. This condition indicated that the rat was on estrus (under influenced of progesterone). In the pregnant rats, all four rats have the highest and distended secretory cells which were protruding into the lumen. This condition was observed at the body of uterus. However, at the area of fetus attachment, the cell was smaller in size but it was still bulging and protruding into the lumen. The finding in this study was different from the results by Laushova (1999), in which he found that the 14 days old rats that have been treated with estrogen have tall secretory cells and protruding into the lumen. However, according to Johnson and Foley (1974), the secretory cell of animal under influenced of progesterone will appear 'balloon' like bulging and bigger. The occurrence of secretory cells were also observed in this study and it showed that pregnant rats have almost high (++++) to profuse (+++++) of secretory cells in the endometrium whereas the non-pregnant rats have moderate (+++) occurrence of secretory cells in all portions as shown in Fig. 6. Thus, the current result is in agreement with Johnson and Foley (1974).

The uterine glands are functions to synthesis glycogen that is an important source of nutrition for the fertilized ovum (Young and Heath, 2000). In this study, the level of glycogen synthesis was not encountered. The appearance of the uterine glands was observed and compared between pregnant and non-pregnant. The glands of non-pregnant rats were straight, single layer of epithelium and small lumen as shown in Fig. 3. This is an accord with Young and Heath (2000) who reported that uterine gland under influenced of estrogen is fairly sparse and straight. The glandular response to chorionic gonadotrophin infusion is characterized by a marked increase in glandular epithelium and glycogen level (Fazleabas *et al*., 1999). The similar finding was also observed in this study in which the glandular epithelium had increased in number, thus the contents also thicker than non-pregnant.

In this study the occurrence of leucocytes infiltration into the endometrium was observed, scored and compared between pregnant and no-pregnant rats. The scoring system is similar to those that have been describing by Zamri-Saad (1997). The leucocytes cells were divided into two which were the polymorphonuclear (PMN) cells and lymphocytes and macrophages. PMN cells are involved as bacterial defense system. Estrogen and progesterone have various effects on infiltration and function of PMN cell (James *et al*., 1983). Non-pregnant rats have higher average score of PMN cell in the epithelium and lamina propria compared to the pregnant rats in almost all portion of uterus. In pregnant rat, almost all the rats have score of + or absent of PMN cells in the epithelium and ++ or occasional. A study by James *et al*. (1983) also found that those animals that have been increased serum estradiol concentration significantly enhanced the migration of PMN cell into the epithelium. However, animals with high physiological progesterone concentration have increased susceptibility to bacterial infection. Thus, it indicates that animal that is not pregnant are more resistance to infection than pregnant animals and pregnant animal is more susceptible to infection. Frank *et al*. (1983) have also found that rabbits exhibit decreased in phagocytic activity under the influenced of progesterone. The mechanism of this changes in pregnant and non-pregnant animal is not known, but several possibilities have been suggested including (i) increased in uterine blood flow induced by estrogen (Rowsen, 1953), (ii) enhancement of uterine contractility by estrogen (Rowsen, 1953), (iii) depression of PMN

functions by progesterone (Hawk, 1960) and (iv) enhancement of PMN functions by estrogen (Hawk, 1960).
Different from PMN cells, the occurrence of lymphocytes and macrophages cell were higher in pregnant compared to the non-pregnant rats in this study. The similar finding also have been reported by other authors (Herman *et al.*, 1992; Cobb *et al.*, 1995; Hunt *et al.*, 2000; Martinez *et al.*, 2002; Heidi *et al.*, 2002; Tekin and Hensen, 2004; Ulrike *et al.*, 2004 and Dalin *et al.*, 2004) that have found increased in lymphocytes and macrophages number in pregnant and progesterone treated animal. The occurrence of lymphocytes and macrophages in the endometrium of all pregnant rats were ranged from high (++++) to profuse (+++++) at four portions except at the proximal uterine horn. However, in non-pregnant rat the occurrence was moderate. According to Trundley and Moffett (2004), if pregnancy ensues, these lymphocytes continue to increase in number and are found in close contact with trophoblast. Thus, it supports this study in which the occurrences of lymphocytes and macrophages cells were mostly found at the area of fetus attachment. A study by Heidi *et al.* (2002) in porcine also found that lymphocytes infiltration mainly at the attachment site suggesting that it was a localized response to conceptus. The number of lymphocytes of goat reproductive tract is related to the stage of the cycle in which the number of lymphocytes significantly increased in secretory phase (high progesterone) compared to the proliferative phase (influenced by estrogen) (Martinez *et al.*, 2002). This fact suggests a reinforcement of the mucosal barrier in the anatomical region that is exposed to pathogen in a critical period while embryo implantation and development (Martinez *et al.*, 2002). Although the morphological changes of endometrium and modification of leucocytes infiltration are a feature of pregnant uterus, their role in pregnancy is still poorly understood. These modifications may be an important during pregnancy. Thus, a study should be done on increasing the immunity of pregnant animal to non-specific pathogen to reduce the culling rate and pregnancy failure due to infection.

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