Structural study of the vagina of albino rat during the estrous cycle and in the aged rats

Thesis

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Contents

Introduction and Aim of the work

Reproductive process in female mammals was characterized by cyclic alteration in the female tract and in sexual receptivity. The recurrent period of receptivity is called Estrous.

The short length of the estrous cycle of rats makes them ideal for investigating the changes occurring during the reproductive cycle ***(Marcondes, et al., ٢٠٠٢)***.

The estrous cycle in female rats lasts from ٤-٦ days and has four phases named, proestrous, estrous, metestrous and diestrous. These phases could be determined according to the cell types observed in the vaginal smear ***(Sporintz,et al., ١٩٩٩; Hubscher, et al., ٢٠٠٥).***

Several studies were carried on the vaginal epithelium of female rats during the different phases of the estrous cycle. ***Parakkal (١٩٧٤)*** studied the changes in the vaginal epithelium of rats after the administration of exogenous hormones to spayed animals. ***Edwards and Levin (١٩٧٥)*** measured the electrical potential difference in the vaginal epithelium during the phases of the estrous cycle.

Whereas, ***Lamb, et al. (١٩٧٨)*** stated that the human cervix was quite accessible for biopsy, yet the entire human reproductive tract was not easily studied. Accordingly, an

appropriately small animal model aided to study changes in the surface epithelium of the entire reproductive tract by scanning electron microscope.

The population of postmenopausal women growing rapidly. In Canada, there were four million women over the age of fifty and about half of them complained of vaginal discomfort **(Johnston, ٢٠٠٣)**

Reviewing the literatures, few studies were found concerning the cyclic histological changes in the vagina of the albino rat. Accordingly, the aim of the present study was to study and determine the changes occurring in the structure of the vagina of rat during the different phases of the estrous cycle and in aged rats using the light and scanning electron microscopes.

I. Estrous Cycle

Long and Evans (١٩٢٢) mentioned that estrous was a cyclic process as ovulation and the cycle consisted of four phases named proestrous, estrous, metestrous and diestrous. They described rats to be polyestrous reaching the puberty at the age of ٦-٨ weeks when the estrous cycle started and each cycle lasted for ٤-٥ days and the first estrous cycle in rat was typically longer. In young virgin female rats, ovulation did not occur at every estrous. They also stated that estrous was dependent on gonadal hormones, whereas ovulation was dependent on gonadotropin.

Engle and Rosasco (١٩٢٧) stated that sexual reproduction in mammalian species took place after puberty when the female was in special physiological condition known as estrous. In young prepubertal female mice, the vaginal orifice was not visible. The onset of puberty occurred in mice at the age of ٣٥ days.

Young, et al. (١٩٤١) described the hormonal changes occurring during the estrous cycle of female rats. The authors mentioned that proestrous involved the development of the ovarian follicles and the secretion of estrogen (get ready phase), and the estrous, also termed the heat phase was the period of sexual receptivity and ovulation. The authors added that in metestrous the progesterone was secreted from the corpus luteum, while the diestrous was the period of hormonal inactivity between cycles.

Mandl (١٩٥١) described six phases in the estrous cycle of adult white rat named as, early diestrous, diestrous, late diestrous, early estrous, estrous and late estrous. The author examined the vaginal smear histology and used it as an index of ovarian activity, as a test of the potency of various steroid hormones and as a means of correlating the cyclic ovarian changes with those in the accessory reproductive organs and other endocrine glands.

Butcher, et al. (١٩٧٤) stated that various types of hormones controlling the female sexual cycles in mice and rats were similar to that of the human sexual cycle. The authors found that the estrous cycle of rat consisted of four phases, proestrous, estrous, metestrous and diestrous. In mice, the metestrous was further subdivided into two phases, metestrous I

and metestrous II. The authors mentioned that ovulation occurred during the estrous phase after the decrease of the plasma estradiol level and the increase of the plasma lutenizing hormone level in surge. However, ovulation was not followed by menstruation as in human.

Edwards and Levin (١٩٧٥) examined the bioelectric parameters of the vagina during the estrous cycle of rat. They found that, in vivo and in vitro, the magnitude of the transvaginal potential difference was greatest at estrus and smallest at diestrous and metestrous. They recorded a change in the values of vaginal resistance in the different phases of the estrous cycle.

Putti and Varano (١٩٧٩) studied the morphological changes of the uterine and vaginal mucosa, which took place during the estrous cycle in mice. They reported that keratinization of the uterine epithelium was noticeably delayed in comparison to that of the vagina. The later was already completely cornified in late estrus, while keratinization of the uterine mucosa occurred in full estrus. They also mentioned that exfoliation of the cornified layer took place in the uterus before the vagina. Analogous difference was observed in the two organs regarding the mitotic activity, so, the vaginal mucosa showed a higher mitotic rate during late diestrous,

while in the uterus the highest mitotic rate occurred only during full estrus.

Montes and Luque (١٩٨٨) studied the effects of ovarian steroids on the vaginal smears of rats. The authors used for preparing and staining the smear the modified Shorr's staining procedure. They mentioned that the estrous phase of the cycle lasted ٣٠ hours and was characterized by marked cornification of the cells and the absence of leucocytes. The metestrous was a ٦ hours phase and was characterized by the appearance of the leucocytes beside the cornified and the epithelial cells. However, they reported that the vaginal contents during the diestrous phase consistently lacked cornified cells, whereas the leucocytes were very plentiful and the duration of this phase was ٤٨ hours. The proestrous lasted for about ١٢ hours, the vaginal smears were devoid of leucocytes, and the nucleated epithelial cells were predominant.

Robbins, et al. (١٩٩٢) studied the afferent nerve fibers supplying the reproductive organs in female rats during the estrous cycle. They recorded multiunit afferent nerve activity from branches of the hypogastric and pelvic nerves in virgin female rats on different stages of the estrous cycle. They also tested the response of pelvic nerve fibers to vaginal distention and found that the minimal pressure necessary to evoke

response was highest in diestrous and lowest in proestrous. They suggested that the overall response sensitivity of afferent fibers in the pelvic nerve was differentially affected by hormonal variations occurring during the estrous cycle.

Chateau, et al. (١٩٩٦) examined the vaginal keratinization during the estrous cycle of rats as a model for evaluating retinoid activity. They assessed the vaginal keratinization by microscopic examination of unstained smears or Papanicolaou stained vaginal smears. The authors stated that the keratinization process was dependent on the endogenous estradiol secreted between the diestrous and proestrous. The authors injected the rats by different doses of all-transretinoic acid at different periods of the estrous cycle and reported that a single injection of ١٠ mg/kg all-transretinoic acid was able to induce complete inhibition of vaginal keratinization in more than ٨٠٪ of the cases.

Sato, et al. (١٩٩٧) examined cellular proliferation in uterus and vagina of rats during the estrous cycle. They counted the numbers of cells at metaphase in the epithelium and stroma at each estrous cycle. They also examined cell death using DNA fragmentation and the electron microscope. The authors found that the mitotic rates in the luminal and glandular uterine epithelial cells were low at metestrous and estrous. Intense

DNA fragmentation was found in the uterus at metestrous and in the vagina at proestrous and metestrous. The apoptotic index increased in the uterine luminal and glandular epithelial cells at metestrous and estrous respectively. The authors concluded that there was an inverse correlation between cell death and cell proliferation in uterine and vaginal epithelial cells of rats during the estrous cycle.

Gettayacamin, et al. (١٩٩٩) used the vaginal swab to distinguish the estrous from the diestrous phases of the estrous cycle in the *Lesser Bandicoot* rat. They mentioned that vaginal swabs contained a white material during estrous and yellow material during diestrous. The changes in vaginal cytology at each stage of the estrous cycle of the *Lesser Bandicoot* rat were similar to those of the common laboratory rat.

Sporintz, et al. (١٩٩٩) stated that rats owing to their short estrous cycle were used for the examination of cyclic changes in the uterine epithelium. Stages of the cycle in rats predated by vaginal smear cytology were verified through the measurement of hormones relevant to the estrous cycle. Based on scanning electron images they could correlate between the surface changes in uterus and the cyclic variations of blood levels of sex hormones.

Catchpole, et al. (٢٠٠٣) used the microprobe analysis of vaginal epithelial cells shed during the estrous cycle of rat to determine the cellular elements present in pro-estrous, estrous, and post-estrous phases. Vaginal smears were placed on carbon planchettes, fixed by freeze-drying and examined using the scanning microscope with an energy dispersive spectrometer. They also calculated the concentration of Na, Mg, P, S, Cl, K, and Ca (mmol/kg dry weight). They found a significant fall of phosphorus at estrous that correlated with the loss of nuclear and cytoplasmic nucleic acids and nucleoproteins. They reported that potassium concentrations did not exhibit significant change between the successive phases of the cycle.

Serpedin (٢٠٠٣) stated that the estrous cycle included all the changes in reproductive organs between two consecutive estrous phases. The author mentioned that the estrous cycle in mice could be subdivided into five phases as follows: proestrous, estrous, metestrous I, metestrous II and diestrous. The author also stated that the simplest way to determine the estrous cycle was by vaginal smear or vaginal wash. They found that during the estrous phase, the vaginal smear contained mostly cornified epithelial cells while leucocytes were absent. In the other phases of the estrous cycle, the vaginal smear exhibited variable numbers of leucocytes as well as non-cornified epithelial cells.

Soares, et al. (٢٠٠٣) described four phases of the estrous cycle in their studies of the functional melatonin receptors in rat. The authors mentioned that vaginal smears in proestrous had numerous nucleated epithelial cells, some cornified epithelial cells and few leucocytes. In estrous, they described the presence of many clusters of cornified epithelial cells, while in metestrous, they reported the presence of some nucleated and cornified epithelial cells and abundant leucocytes. The vaginal smears in diestrous were characterized by the presence of thick mucous, numerous leucocytes and few epithelial cells.

Yamada, et al. (٢٠٠٣) examined the effect of ١-Bromopropane on the reproductive tract of female rats. In their study, daily vaginal smears were taken to monitor the ovarian cycle pattern and the smears were stained with ٠,٥٪ methylene blue solution and examined under the light microscope. Cycle phases were classified as proestrous, estrous, diestrous I and diestrous II, and the estrous cycle was defined as normal when it showed the four phases within four to six days.

Hubscher, et al. (٢٠٠٥) used a quantitative method for assessing the phases of the estrous cycle of rat. They stated that these phases could be determined by examining samples of cells obtained from the surface of the vaginal epithelium. The authors stained the vaginal smears by modification of the

original Papanicolaou (PAP) stain. Whereas the original PAP staining procedure was a regressive method, where tissue was overstained followed by removal of excess stain, yet the authors in this study used a progressive staining method where the samples were stained until the desired color intensity was reached.

II. The structure of the Vagina

Young, et al. (١٩٤١) described the vagina as a short muscular canal leading from the uterus of female rat to the outside of the body and serves as a birth canal. It also acts as an orifice for the acceptance of sperm during mating. When born, females have a vaginal closure membrane (vaginal plate) that ruptures on its own by the time the offsprings reached ٣٣-٤٢ days of age.

Parakkal (١٩٧٤) examined the changes in the vaginal epithelium of normal cycling rats and after the administration of exogenous hormones as estrogen and progesterone to spayed rats using the scanning electron microscope. They reported that the keratinization of the vaginal epithelium was related to the