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## The Role of FSH, LH, Estradiol and Progesterone Hormone on Estrus Cycle of Female Rats

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### Abstract

The estrous cycle is a recurrent process that describes changes in reproductive hormone levels caused by ovarian activity under the influence of pituitary hormones. Estrous cycles are characterized by morphological changes in ovaries, the uterus and the vaginal cells. The length of the estrous cycle and the frequency of the reproductive cycle in each organism are different. In mice, lasts for about 4-5 days.

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The estrus cycle consists of four phases, namely proestrus, estrus, metestrus and diestrus. During this period, the vaginal mucosa undergoes tremendous structural changes and is affected by hormones such as FSH (stimulates the growth and development of ovarian follicles), LH (regulates estrogen and progesterone hormones), progesterone and estradiol. Previous studies showed that in proestrus phase serum estradiol concentrations higher than all other estrus stage ( $p$ -value  $< 0.001$ ). FSH concentrations tend to be higher during proestrus. During estrus, serum estradiol concentrations were significantly lower than proestrus ( $p$ -value  $< 0.001$ ) higher than metestrus ( $p$ -value = 0.005). Admission to metestrus coincides with the continuous rise in levels of the progesterone hormone. Estrus, 17- $\beta$ -estradiol decreased and peak prolactin levels. The Serum estradiol increased from metestrus through proestrus (ANOVA  $p$ -value  $< 0.001$ ). During metestrus, serum estradiol concentration was significantly lower than proestrus ( $p$ -value  $< 0.001$ ), estrus ( $p$ -value = 0.005) and diestrus ( $p$ -value = 0.034), LH concentrations tend to be higher during proestrus and metestrus. The concentration of serum LH and FSH did not differ statistically across the whole of the estrous cycle (Kruskal-Wallis test  $p$ -value = 0.333 and 0.643, respectively). Serum estradiol concentrations were lower than proestrus ( $p$ -value  $< 0.001$ ) and higher than metestrus ( $p$ -value = 0.034). In this article will be discussed explanation of each hormone and its effect to the estrus cycle, especially changes in vaginal cells.

**Keywords:** FSH; LH; Estradiol; Progesterone; Estrus Cycle; vaginal cells.

## 1. Introduction

The estrous cycle (British spelling, oestrous) is the main reproductive cycle of other species females of non-primate vertebrates, for example rats, mice, horses, pig have this form of reproductive cycle. Estrous cycles are characterized by morphological changes in ovaries, the uterus and the vagina [39]. Estrous cycle is the reproductive system in most mammals, reproductive hormones cause the cycles to start after puberty in sexually mature females. The mean duration of the estrous cycle was 4-5 days for 60%-70% of female rats. The short length of the estrous cycle of rats makes them ideal for investigation of changes occurring during the reproductive cycle. The rat has been elected as the main animal model in several studies involving reproduction. Progress in our understanding of the complexity of GnRH action on the pituitary and the various mechanisms involved in differential LH mediation and FSH biosynthesis and secretion in gonadotropin, is still being discussed. Gonadotropin, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), are produced exclusively in gonadotropin cells of the anterior pituitary and secreted into the blood where they regulate steroidogenesis and gametogenesis in the gonads. The synthesis and secretion of LH and FSH are influenced by GnRH. The synthesis and secretion of LH and FSH are arranged either positively or negatively by peptide gonadal and steroids [1]. The GnRH receptor suppression and serum gonadotropin with combined estradiol and progesterone treatment [2]. These hormones play an important role in the reproductive cycle. The reproductive cycle is a repetitive process that occurs in the reproductive system of adult female animals that exhibit changes in certain reproductive organs. These organs are such as ovaries, oviducts, uterus, and vagina. The reproductive cycle in mammals (primates) is called the menstrual cycle, whereas the reproductive cycle in non-primates (rats) is called the estrous cycle [3]. The estrous cycle is a recurrent process that describes changes in reproductive hormone levels caused by ovarian activity under the influence of pituitary hormones. Changes in reproductive hormone levels further lead to structural changes in the reproduction tract network. The length of the estrous

cycle and the frequency of the reproductive cycle in each organism are different. In mice, the estrus cycle lasts for about 4-5 days [3,4,5,6], and during this period, experiencing vaginal mucosa remarkable structural changes [7,8].

## **2. Discussion**

II.A. The types of hormones that play a role in the estrus cycle are :

### *FSH*

As a kind of gonadotropins, FSH is secreted by the basophilic cells of the anterior pituitary and have important roles in gonadal hormone production and regulation of the reproductive process [9]. The FSH receptor is a G-plus receptor protein, which is mainly expressed in Sertoli Testicular cells and ovarian granular cells [10]. As an employer FSH hormone in the hypothalamus-pituitary-gonadal (HPG) axis is, GnRH is secreted primarily from hypothalamic neurons into the capillary plexus of the median eminence and reach the anterior pituitary, combined with GnRH receptor to regulate the secretion of FSH [11]. The Follicle-stimulating hormone (FSH) stimulates the growth and development of ovarian follicles, where the development of ova or egg cells and promote the secretion of estrogen by the ovaries. Maximum FSH is the best predictor of FSH-based ovarian reserve [12]. The FSH receptor is expressed only in ovarian granulosa cells [13]. FSH acts primarily to promote follicular growth, granulosa cell proliferation, aromatization of androgens to estrogens, and LH receptor expression. LH is also necessary for follicle growth, particularly at the late stages, and, as noted below, induces ovulation [40]

### *LH*

LH in conjunction with FSH stimulates these internal cells of follicle to secrete oestrogen. The LH causes preovulatory enlargement of follicle. The gonadotrophins luteinising hormone (LH) is essential for the completion of follicular maturation and development of mature preovulatory (Graafian) follicles. The Luteinizing hormone (LH) formation of the hormone-producing corpus luteum in the ovaries after ovulation and regulates the hormones estrogen and progesterone [14].

### *Estrogen*

The steroid hormones involved in the estrus cycle produced by the ovaries are estrogen and progesterone. Estrogen is a steroid compound that acts as a reproductive hormone in females. The hormone is responsible for the growth and development of the vagina, uterus, and vital organs for ovum transport, zygote maturation, and the concept of zygote implantation. In addition, the hormone is involved in endometrial thickening and in the setting of the estrus cycle. Therefore, estrogen content is much higher in the body of female rats that are at the age of fertile [15]. Fertile period marked by the release of the female egg cells mature through the events of ovulation [16]. During this period, the hormone estrogen achieve a maximum level and then declined rapidly. After ovulation occurs, low levels of estrogen will be replaced by an increase in progesterone levels. The 17- $\beta$ -estradiol increase indirectly stimulates the hormone gonadotropin-releasing neurons in the hypothalamus and

septum which, in turn, activates responsive cells in the anterior pituitary to release the luteinizing hormone and follicle-stimulating hormone into the circulation [17,18]. Changes in vaginal mucosa histological and cytological sex steroid hormone dependent. The main hormone that drives vaginal mucosal changes is estradiol [19].

### *Progesterone*

The Progesterone is a steroid hormone involved in the estrus and pregnancy cycles, progesterone includes the progestagen hormone class. The Progesterone is produced by the corpus luteum in the ovaries after ovulation and in the adrenal glands located near the kidney, as well as inside the placenta during pregnancy [15]. Increased progesterone levels indicate ovulation has occurred and progesterone levels will peak in midluteal phase of the cycle. Fluctuations in levels of these hormones are a response to the workings of pituitary hormones in the ovary organs [3]. The Progesterone is responsible for preparing the reproductive system for implantation of the zygote. This suggests that progesterone present in preovulatory plasma may trigger sexual behavior in some species. The Progesterone has a dominant role in regulating the estrus cycle [15], levels of progesterone in the blood of mice at the beginning of the estrous cycle is less than 5 ng / ml, after ovulation levels of more than 5 ng/ml [20]. The Progesterone is considered to represent identified ovulation event, progesterone will only be secreted through a body formed after ovulation. After ovulation occurs, progesterone levels begin to increase, and continue to increase until it reaches the maximum amount [3]. The Fluctuations in progesterone levels throughout the cycle to give a complete picture of the hormone progesterone profile. The level of progesterone at the time of non-estrus increased four times compared to the time of estrus [21]. The role of progesterone vaginal mucosal epithelium of the less obvious but records indicate that they may have a mild effect of estradiol antagonistic effects on the vaginal mucosa [22].

### **II.B. The estrus cycle**

The estrus cycle is divided into two phases, the follicular phase and the luteal phase. The follicular phase is the formation of follicles until cooking, while the luteal phase is the phase after ovulation, then the formation of the corpus luteum and until the start of the cycle. The estrus cycle consists of four phases, namely proestrus, estrus, metestrus and diestrus. Each phase in the cycle is determined based on the epithelial cell form on vaginal cytology observation [6,23]. The cytokines vaginal cytology reflect underlying endocrine events [24,25]. The vaginal exfoliative cytology can be used as a general guide to determine the desired endpoint estrus stage, and definitive confirmation was obtained from the evaluation stage of estrus vagina [26] Vaginal exfoliative cytology is an established tool for the assessment and classification of the rat estrous cycle phases. This stage [19,22,23,25,27,28,29] then absolute, and no other diagnostic steps are taken to ensure that the vaginal cytology results in a correct classification of the estrous cycle [30,31]. Correlating the level of estradiol, and serum gonadotropin with an estrus cycle stage of a single morning blood sample is sufficient to show serum estradiol changes throughout the estrous cycle [26]. Sampling every day for serum levels of estradiol by phlebotomy can not be regarded as a good tool to monitor the stages of estrus in mice, because it can lead to morbidity and mortality unwarranted [32].

The estrous cycle lasts four days and is characterized as: proestrus, estrus, metestrus and diestrus, which may be determined according to the cell types observed in the vaginal smear. The following are the types of phases in the estrous cycle :

#### *Proestrus*

The proestrus phase of the estrous cycle corresponds to the human follicular phase of the menstrual cycle [24]. During proestrus vaginal smear contains many nucleated epithelial cells and some leukocytes [27,28,29,33]. In proestrus phase serum estradiol concentrations higher than all other estrus stage (p-value <0.001). FSH concentrations tend to be higher during proestrus [26].

#### *Estrus*

During estrus there is characterized the cornification of the cells and the loss of leukocytes. Papinal vaginas are characterized by the almost exclusive detection of irregularly shaped squamous epithelial squamous cells often in clumps [27,28,29,33]. Under the influence of estradiol vaginal mucosal epithelium stratifies and become cornified. On the other hand, the withdrawal of estradiol leads to a widespread desquamation of the mucosal epithelium to the vaginal lumen. During estrus, serum estradiol concentrations were significantly lower than proestrus (p-value <0.001) higher than metestrus (p-value = 0.005). Admission to metestrus coincides with the continuous rise in levels of the progesterone hormone. Estrus, 17- $\beta$ -estradiol decreased and peak prolactin levels [26].

#### *Metestrus*

In the course of the metestrus, the sloughed cornified coating and mucosal invasion by leucocytes occurs, cell types present in the vaginal swab during this fragmented stage, the cornified epithelial cells and the small dark leukocyte sodium [27,28,29,33]. The Serum estradiol increased from metestrus through proestrus (ANOVA p-value <0.001). During metestrus, serum estradiol concentration was significantly lower than proestrus (p-value <0.001), estrus (p-value = 0.005) and diestrus (p-value = 0.034), LH concentrations tend to be higher during proestrus and metestrus [26]. As progesterone levels begin to rise and there was a small spike in the 17- $\beta$ -estradiol levels in response to the activation of the corpus luteum [34,35,36] causing regression of the corpus luteum following a sharp decline in the level of progesterone [37,38]. Finally, go to diestrus in mice occurs and levels of circulating progesterone peaks [34].

#### *Diestrus*

In the diestrus phase the contents of the vagina consistently lack of cornified cells and leukocytes predominate in the smear. The frequency of the cornified epithelial cells decreases and the nucleated epithelial cells begin to be detected just before the transition to the proestrus [27,28,29,33]. The concentration of serum LH and FSH did not differ statistically across the whole of the estrous cycle (Kruskal-Wallis test p-value = 0.333 and 0.643, respectively). Serum estradiol concentrations were lower than proestrus (p-value <0.001) and higher than metestrus (p-value = 0.034) [26].

### **3. Conclusion**

Estrous cycle is the reproductive system in most mammals, reproductive hormones cause the cycles to start after puberty in sexually mature females. The mean duration of the estrous cycle was 4-5 days for 60%-70% of female rats. The rat has been elected as the main animal model in several studies involving reproduction. However, there are scarce and conflicting data related to its estrous cycle. The short length of the estrous cycle of rats makes them ideal for investigation of changes occurring during the reproductive cycle. The estrous cycle lasts four days and is characterized as: proestrus, estrus, metestrus and diestrus, which may be determined according to the cell types observed in the vaginal smear. The estrous cycle is a recurrent process that describes changes in reproductive hormone levels caused by ovarian activity under the influence of pituitary hormones. Gonadotropin, Follicle-stimulating hormone (FSH) stimulates the growth and development of ovarian follicles, the site of ovum and promotes estrogen hormone secretion by the ovaries and Luteinizing hormone (LH) formation of the hormone-producing corpus luteum in the ovaries after ovulation and regulates the hormones estrogen and progesterone. Histological changes and cytology of the vaginal mucosa depend on the sex steroid hormone. The main hormone that drives vaginal mucosal changes is estradiol. The role of progesterone in the vaginal mucosa epithelium is less clear but the data suggest that they may have mild antagonistic effects of estradiol effects on the vaginal mucosa.

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