EFFECT OF SEXUAL BEHAVIOR AND PHEROMONES OF OVARIECTOMIZED FEMALE ON TESTOSTERONE PLASMA LEVELS IN MALE WISTAR RAT

***Seyedeh Masumeh Honarmand**,*
Animal Physiology (M.Sc.), Province Health Center, Busher Medical University, Busher, Iran
(Corresponding: Seyedeh Masumeh Honarmand, honarmand1342a@gmail.com)
Heydar Aqababa,
Ph.D., Department of Biology, Arsanjan Branch, Islamic Azad University, Arsanjan, Iran
Shidokht Ostovani,
Ph.D., Department of Biology, Arsanjan Branch, Islamic Azad University, Arsanjan, Iran
Hamideh Eftekhari,
Ph.D., Department of Biology, Arsanjan Branch, Islamic Azad University, Arsanjan, Iran

Abstract. Background: Pheromones are responsible for sexual and social behavior among members of the same species. In this research, the goal was to examine the changes of testosterone plasma levels in male Wistar rats that have been faced with sexual behavior and pheromones of ovariectomized female .**Materials and Methods:** In this experimental-interventional study 48 rats were divided into three groups (control , experimental 1and 2), each group contained 8 male and 8 female. After 24 hour, control and experimental 1 groups remained in their cages; but male rats in experimental 2 group, were separated from their pregnant pair and as strange male rat were placed in the neighborhood with another female pregnant of the same group in the special cages that just moved volatile pheromones of female to male. (Experimental 1, 2 groups female rats, 17th day postpartum were ovariectomized). In the first day from control group mail rats and10th day of pregnancy, 14th and 24th days of postpartum, blood samples were collected from all male rats. Data based on editing 19 SPSS statistical program and one-way ANOVA statistical methods were analyzed. The significance level For results ($P \geq 0.05$) significant was considered. **Results:**. The testosterone plasma levels had significant decrease among experimental 1, 2 groups male rats in 24th day of postpartum. **Conclusion:** These findings suggest that female rats non-volatile pheromone's (when the ovaries are active) can stimulate sexual behavior in male rats.

Keywords: ovariectomy, rat, sexual pheromones, sexual behaviors, testosterone.

Introduction. Pheromones are chemical compounds that naturally exist in all insects, animals and humans. The word "pheromone" was introduced in 1959 by Peter Karloson & Martin Lusche based on the Greek words "hormone" and "pherein" (hormones and fetuses), meaning transference and incitement. Pheromones or Ectohormones are chemical messenger molecules that are transmitted outside the body and capable of producing responses such as physiological and behavioral changes (1-5). Pheromones are mainly divided into two categories: 1) releaser pheromones that generate short-term behavioral changes and act as attractant or repulsive; 2) primer pheromones that cause long-term changes in behavior or evolution by activating the hypothalamus-pituitary-adrenal axis [6].

In terrestrial environments, chemical messages can be volatile or non-volatile. Accordingly, Dermatoglycans have two distinct functional and anatomical olfactory systems: the main olfactory system, whose receptors are near the nasal passage and have the strongest response to the air containing pheromones, and transmit the volatile messages to stimulate the hypothalamus, and the Vomeronasal organ, located in the anterior nasal cavity (7), that is mainly responsible for the process of recognition of non-invasive pheromones (8). Mammal pheromones control countless social behaviors and regulate levels of hormones (9).

By Whitten Effect, when female mice are in direct contact with male urine or smell, they ovulate 3-4 days later, which are physiologically fertile and sexually sensitive to the opposite sex (10 and 11). What is the role of gonads in female sex? Do female volatile pheromones alone increase the level of male testosterone and sexual stimulation? Can the results on how pheromones function in rodents be generalized to humans? The purpose of this experimental study was to investigate the effect of sexual behaviors and ovariectomy female sexual pheromones on plasma level of testosterone hormones in male rats.

Methodology. *Purchasing and keeping animals*: 24 female rats and 24 male Wistar rats in a weight range of 180±20 g were purchased from Pasteur Institute of Karaj and were kept in the animal room of the Islamic Azad University of Arsanjan Branch. The temperature of the animal room was in the range of 22-28 ° C, the light conditions were 12 hours of darkness, 12 hours of brightness and the ventilation conditions were also well-established. 16 typical cages and 8 special cages were also used. These cages consisted of two separate compartments separated by a metal wall and the odor of the female rats was transferred to the male rats through the ventilator embedded in the middle of cage (Fig. 2).

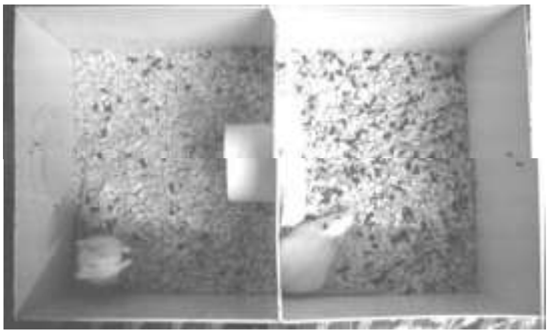

Figure 1: An overview of the special cage

As can be seen, the ventilator embedded in the middle of the cage on a metal wall causes the smell of urine and sweat of the female rat on the right side to be transferred to the male rat on the left side (16).

Grouping and work steps: To synchronize the female rats' cycle and prepare them simultaneously to mating, they were placed for three days in cages where the adult male rats were kept until the volatile pheromones of male rats that were left in the cages reach the female rats and the female rats become ready for mating. Then, we divided male and female rats into 3 groups of control, experimental 1 and 2. First, zero time blood sample was taken from 8 male rats of the control group (1.5 cc per male rats). In the next step, the rats of each of the three groups were placed in a typical cage in pairs, one adult male rat and one adult female rat for mating. Twenty-four hours after mating of each pair in a typical cage, experimental 2 male rats were separated from their pregnant pairs. They were placed in a cage as a stranger, adjacent to a pregnant woman from the same group. On the 10th, 14th and 24th days after pregnancy, blood samples were taken from the heart of all male rats. For blood sampling from the heart, male rats were muttered as in semi-conscious state with ether. Then, the animal was fixed on the fixed tray and the strongest heart rate (ventricle) was found using the sense of index finger or thumb. After that, 1.5 cc of blood sample was slowly taken using the 2 cc syringe and G 25 Nidel at 35 degrees. After removing Nidel from the syringe, the blood was drained slowly in the Gamma tube, adjacent to the inner wall of the tube. After separating the serum, the specimens were stored in screw cap microtubes at -23 ° C in the freezer until the time of testosterone level testing (80 samples in 4 phases of blood sampling).

Female rats of the experimental groups were ovariectomized on day 17 after delivery. Bilateral back-side ovariectomy, invented since 2010, was done. For the anesthetization of rats, a combination of ketamine (50mg / kg) and xylacin (10mg / kg), produced by Iman and Saba Company of Shiraz, was used. After the anesthetics of the female rats, the skin was shaved in the ilium area under the broad ligament. In the middle of the most inferior point of ilium, the incision was diagonally as large as 1.5 cm, and the skin of the back-side areas of the abdomen and diagonal muscle was opened. In the abdominal cavity, peritoneum and muscle were removed. After identifying the ovary and horn of the uterus, under the ovary, the uterine horn was ligatured to completely separate the ovary in one cut. The uterine horn was returned to the abdomen and the peritoneum, muscle and skin were sutured with X matrix and sprayed with tetracycline fluid. All ovariectomized rats used 4 mg/l cephalexin solution instead of water up to 5 days after surgery to prevent infections.

Hormonal test: Hormone assay was performed in all blood samples by Total Testosterone kits purchased from Saman Tajhiz Noor Co. by ELISA method. The results were analyzed statistically.

Statistical methods: The results of the data were analyzed using SPSS-19 and one-way ANOVA and investigated by tuckey post hoc test. Significance level was considered to be $P \leq .05$.

Findings. The results of the statistical analysis of the research data are as follows.

A) Evaluation of the effects of female rat pregnancy on the secretion of testosterone hormones in pair and stranger male rats on day 10 of pregnancy

According to Table 1 and Figure 1, results of blood sampling in the first turn among male rats of control and experimental groups 1 and 2 showed a significant decrease in the concentration of testosterone in the experimental group 2, in which stranger male rats were placed adjacent to the pregnant female rat in a special cage, in comparison to male rats in experimental group 1 and control group. However, there was no significant decrease in the concentration of testosterone in male rats of experimental group 1 compared to male rats of control group.

B) Evaluation of the effect of delivery on the secretion of testosterone hormones in pair and stranger male rats on day 15 after childbirth

According to Table 2 and Figure 2, this second stage of blood sampling (second turn) showed a significant increase in the concentration of testosterone in male rats of experimental group 2 than in experimental 1 and control groups. However, there was no significant difference in the concentration of testosterone in the experimental group 1 compared to the control group. This result indicates that the testosterone concentration of stranger male rats in a special cage in the vicinity of the volatile pheromone of non-couples and stranger children was significantly higher than that of the male rats that were placed alongside the female pair and their offspring.

C) Evaluation of the effect of ovariectomy on the secretion of testosterone hormones in pair and stranger male rats on day 24 after childbirth

According to Table 3 and Figure 3, there was a significant decrease in the concentration of testosterone in experimental groups 1 and 2 compared to the control group in the third turn blood sampling. However, there was no significant difference between the two groups (experimental 1 and experimental 2) for the average testosterone concentration of male rats in the final blood sampling one week after the ovariectomy of the female rats.

Discussion. Male hormones include gonadotropins (LH and FSH[64]) produced by the anterior pituitary and 5-α-D hydrotestosterone produced by gonads (12). Accordingly, the concentration of testosterone of male rats of experimental group 2 (stranger males) that received volatile pheromones (smell of sweat and urine) of the female rats only through the central ventilation of the cage wall was significantly lower on the 10th day of pregnancy in comparison to the concentration of male rats' testosterone in the control and experimental group 1 that were placed next to their pair and exposed to non-volatile pheromone in addition to volatile pheromone, and had the possibility of using vision sensing, hearing, touching, licking, and intercourse. Obviously, on the tenth day of pregnancy of the female rats, whose embryos are implanted in the two horns of the womb and the pair is formed, estrogen and progesterone secretion of the ovary is increased due to the presence of pair gonadotropins in addition to pituitary gonadotropins. In addition, vaginal discharge containing pheromones that are non-volatile and controlled by the ovaries is increased.

According to Kazushige's research on vertebrates, peptide and protein pheromones were mostly identified among amphibians and rodents. Major urinary proteins were largely found in urine of adult male rats (13). The results of Liberles and StephenD's research showed that urine of female rats attracted male rats (14).

According to Bliss and Baker's theory in 1991, female pheromone stimuli during intercourse can affect male neuroendocrine responses and guarantee the occurrence of reproductive behavior during intercourse [15]. Research results of Aqababa et al. (2007) showed that testosterone levels increased during sexual intercourse compared to when male rats received only female pheromones (16). The results are consistent with the results obtained in the present study. In a study by Peter et al. (2001), it was found that the plasma levels of testosterone were lower in males having children than in unmarried males and males without children (17). Based on the findings of Aqababa et al. (2007), female sex pheromones and various sexual and paternal behaviors directly affect the plasma levels of testosterone and, as a result, reproductive activity of male rats. It was also observed that the plasma level of testosterone hormones again increased when the father rat was placed along the stranger children (male or female), which itself reflected the interaction of pheromones released from children on the endocrine system of the father (16). According to the results of the research, we find that all of the above results confirm the result of the present study that in the control and experimental groups where a male parent was along with his pair and children, the emergence of parental feelings decreased the concentration of testosterone in male rats of both groups. However, in the experimental group 2, where stranger male rats received volatile pheromones of mother rats and infants born of mating of other male rats with mother rats through a ventilation inserted in the cage wall, there were changes in the endocrine system and, as a result, a significant increase in the concentration of testosterone in them.

In evaluating the intensity and smell of vaginal discharge in a full cycle of menstruation, Dati et al. found that the most intense and most pleasant odor of vaginal discharge was in ovulation (13). Regarding the study of the mechanism of action of female sex hormones, a study by Iman Lisa and Stacy has shown that there is a direct relationship between levels of motor activity, changes in secretion of dopamine, estrous cycle, and estradiol and progesterone levels (18, 19).

Shanmagham Achimerman et al. (2010) showed that the natural urine of the female rat contains a mixture of isocrothylhydrazine, 4-methyl-2-heptanone and azolone during the period of pre-estrus prevalence. Also, the 1-iodo-2-methyl undecane combination is observed in the urine of female rats during pre-estrus and estrus periods that is estrogen-dependent and acts as a sexual pheromone. For this combination, effects such as sniffling, licking, and self-cleaning have been recorded in the animal before mating behavior. None of the estrus-specific compounds and 1I2MU were detected in urine of ovariectomized rats. Based on the pre-mating behavior analysis, male rats showed the highest sexual preference to 1I2MU. Female rat's vaginal discharge is detected by the MOE[65] of male rats. The MOE helps the animal identify its pair from other females. They are also recognized by the VNO, and they help the male rat select pairs and apply certain behaviors during inactivation. In many species, it was observed that the diagnosis of sex pheromones by contact with the opposite sex, through VNO, would increase the amount of hormone lutein, and consequently, testosterone (20). The results of this study are in line with the findings of the study on monkeys, rats and mice. These findings indicate that in male rats' blood sampling one week after female rats' ovariectomy, their testosterone concentrations had significant decrease in the experimental group 1, in which they were placed in conventional cages by their pairs, and the stranger male rats in the experimental group 2 that were placed in specific cages and only received an ovariectomized female rat's volatile pheromone. The difference in testosterone concentration in the experimental group 1 and 2 was not significant.

The sense of smell is important because it is an excitatory system that attracts attention to events and important environmental changes. Humans have the ability to store memories of smell and to create arousing interest or aversion to smell. The olfactory messages inspire emotional reactions. This is probably due to the fact that olfactory receptors send predictions to the neocortex for conscious processing and to the limbic system for their emotional processing (22, 21).

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[64] Lutein Hormone & Follicle Stimulating Hormone

[65] Main Olfactory Epithelium

But the results of this study cannot yet be attributed to humans, and there is no strong laboratory evidence. A common problem is the small size of the samples and the small amount of their valuable effect. For humans, it is necessary to use very precise methods that are employed to prove pheromones in other species (23).

Conclusion. Given that the mother rats were undergone ovariectomy at 17 days postpartum, blood sampling from male rats in experimental groups 1 and 2 after a week that they received volatile pheromone of ovariectomized female rats showed a significant reduction in serum testosterone concentrations in male rats.

Ultimately, the importance of pheromone in creating a definite physiological response and in influencing attitudes in our lives as a general matter remains an unanswered question. As a result, revealing the code of smells and the way that humans feel them needs time and effort.

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