



# Impact of Sexual Deprivation on Sexual Behavior and Some Reproductive-Endocrinal Functions in Albino Rats

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## ABSTRACT

**Objective:** To study the effect of stress on the sexual behavior and its pathophysiological effects on some reproductive and endocrine functions in albino rats.

**Methods:** One hundred and twenty albino rats were included and divided into a control group and three experimental sub-groups, which were subjected to sexual stress. Female rats were investigated for the cytological changes in the phases of the estrous cycle. All rats were observed for behavioral changes throughout the experiment. Histopathological examination of the thyroid, testes and ovaries and the assessment of thyroid and gonadal hormones in the sera of control and experimental rats were performed.

**Results:** Cytological examination revealed stopped estrous cycle in the diestrous phase in all female rats. Thyroid hormones revealed a decrease in the levels of triiodothyronine and thyroxine; however, non-significant changes were detected in the thyroid-stimulating hormone level in experimental rats compared to the controls. Gonadal hormones revealed a great discrepancy in their levels among both sexes.

**Conclusions:** The results of the present study show that sexual excitation is one of the stressful factors affecting sexual behavior, hypothalamic-pituitary-thyroid and hypothalamic-pituitary-gonadal axes as well as sex organs with secretory functions. Therefore, it is considered as a socio-pathological factor that needs more specific studies to further clarify its effects.

**Keywords:** Sexual deprivation, Sexual behavior, Thyroid hormones, Gonadal hormones

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## 1. Introduction

Individual physiological needs include those for oxygen, water, food, sex and avoidance of pain. Generally, all needs are essential for homeostasis or survival (1). Under stressful conditions, or when survival is threatened, the organism could regress to a lower level of needs (2). For example, sex, water or food deprivation causes regression to a lower level of needs to satisfy such a deprivation. In animals, sexual behavior is mainly considered as a physiological process, while it has physiological, psychological and social aspects in humans (3).

Physiological basis of sexual behavior is usually studied in animals because it is difficult to carry out sex deprivation studies in humans. However, sexual behavior studies in animals are defective as they mostly illustrate the physiological aspect but lack the social and psychological aspects, which form with the physiological aspect a complex triad in humans. Living organisms facing stressful situations, such as sexual dissatisfaction, usually suffer from pathophysiological disturbances (4). Therefore, complicated socio-financial responsibilities that postpone marriage as a means of sexual satisfaction among a wide sector of youth in the society (5) may play an important role in health and socio-pathological disturbances. Moreover, behavioral stress was reported to prevent the organism from achieving normal reproductive efficiency (6). Various factors can disrupt the female reproductive cycle, resulting in sub-fertility. For instance, psychological changes in rats exposed to hind-limb suspension were found to be associated with estrous cycle lengthening due to prolonged diestrus and reduced estrus (7).

Stressful stimuli affect the activity of the hypothalamic-pituitary-adrenal (HPA) axis in rodents, domestic species and primates (8). They commonly lead to a marked and persistent in-

crease in glucocorticoid secretion, reduced fertility and suppressed gonadotropin secretion. Although the cellular and biochemical basis for the anti-gonadal effect of stress has not been clearly understood, it was found that glucocorticoids act at the hypothalamic and pituitary levels to decrease gonadotropin secretion (9). The stress-induced secretion of adrenal glucocorticoids is particularly significant because these steroids can affect both synthesis and secretion of gonadotropins. In addition, corticotropin-releasing hormone and adrenocorticotropin may play an additional role in the regulation of hypothalamic-pituitary-gonadal (HPG) axis (6).

The present study was designed to determine the impact of sexual deprivation on sexual behavior and its pathophysiological effects and on some reproductive-endocrinal functions in albino rats.

## 2. Methods

### 2.1. Selection and housing of rats

One hundred and twenty albino rats included in the model of the present study were purchased from Nile Pharmaceuticals (Cairo, Egypt): 96 mature albino rats (48 males and 48 females) aged four months and with a body weight of 160–200 gm and 24 immature rats (12 males and 12 females) aged 21–22 days and with a body weight of 30–60 gm. The immature rats were changed every 10 days for not to reach maturity. They were maintained on a standard diet of commercial rat chow and tap water *ad libitum*. The rats were housed in wire mesh cages with plastic floors; every two rats in a 25 × 25 × 30-cm cage. Before experiments, the rats were adapted to laboratory conditions for two weeks in a room free of any possible cause of stress, such as noise or bad ventilation, under the supervision of well-trained authorized personnel. All the experiments were conducted in accordance with the



standard animal ethics and the study protocol was reviewed and approved by the ethical committee of faculty of medicine, Sana'a University.

## 2.2. Experimental design

The rats were randomly allocated to two main groups: A control group (Group I) of 24 mature rats (12 males and 12 females) that were kept in a separate room from the experimental group to avoid affection of their pheromones on the experimental rats and an experimental group (Group II) of 96 rats comprised of 72 mature (36 males and 36 females) and 24 immature (12 males and 12 females) rats. The experimental rats were then randomly sub-divided into three sub-groups as follows:

1. Sub-group IIa: Twenty-four mature rats (12 males and 12 females) were kept in 12 metal cages divided into two compartments by a fixed wire meshwork. In each cage, one male and one female rats were placed in the separate compartments of the cage to allow the visual and olfactory arousal of the male by the female genital tract pheromones as well as the female arousal by the male odor.
2. Sub-group IIb: Thirty-six rats (12 adult males, 12 females and 12 immature females) were kept in 12 metal cages. In each cage, one mature male and one immature female were placed in one compartment separated from one mature female in the other compartment.
3. Sub-group IIc: Thirty-six rats (12 mature males, 12 mature females and 12 immature males) were kept in 12 metal cages. In each cage, one mature female and one immature male were placed in one compartment separated from one mature male in the other compartment.

## 2.3. Sexual stimulation and observation of rats

The experimental rats were subjected to the fol-

lowing procedures:

1. Induction of sexual arousal of males by the pheromones of females and of females by the odors of males.
2. Observation of vaginal opening (VO), where each female was observed daily for VO from the postnatal day 21. The age and body weight of the female were recorded when the VO was first observed.
3. Observation of rat sexual behaviors, including trial to perform intercourse, displaying coital position, body contact, oral stimulation, etc.
4. Determination of estrous cycle by vaginal smear examination as described previously (10). This procedure was repeated daily for 12 cycles.

## 2.4. Blood sampling

At the end of the experiments, rats were anesthetized using light ether anesthesia. Then, whole blood was withdrawn from each rat into a glass tube by retro-orbital puncture and allowed to clot. Clotted blood samples were centrifuged at 3000 rounds per minute for about 15 minutes, and sera were then collected using an automatic micropipette into special clean dry serology tubes and stored at -20°C until assayed.

## 2.5. Determination of hormone levels

The levels of triiodothyronine (T3), thyroxin (T4) and thyroid-stimulating hormone (TSH) were determined to assess thyroid function using commercial kits (DRG International Inc., Germany) according to the instructions of the manufacturers. In addition, the levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, estradiol, progesterone and prolactin gonadal hormones were determined to assess the reproductive function using commercial kits according to the instructions of the manufacturers.



## 2.6. Pathophysiological study

After blood withdrawal, each rat was anesthetized and then killed by cervical dislocation. The abdominal cavity was opened, and the thyroid gland, testes and ovaries were excised. The tissue samples obtained were kept in 10% formalin solution. Paraffin blocks were made for the tissue samples, and different sections at multiple levels were prepared. Smears of tissues were stained with hematoxylin and eosin and examined using the 40x objective of a light microscope. The immature rats were considered as a factor of stress.

## 2.7. Statistical analysis

Data were analyzed using CoStat statistical software (CoHort Software, USA). Quantitative data were presented as means  $\pm$  standard deviations. One-way analysis of variance (ANOVA) was used to compare between the means of the studied groups according to (11). *P*-values  $<0.05$  were considered statistically significant.

## 3. Results

### 3.1. Description of rats' behaviors

Control rats displayed an average degree of motor activity inside their cages. On manipulation, they tried to escape and showed slight aggression towards the person dealing with them, with no interspecies aggression. In contrast, experimental rats showed typical signs of stress over the whole experiment. These behaviors changed irregularly during the experiment. As regards the sexual behavior, stressed rats tried to overcome and cut the barriers between the compartments to creep over the mesh that interferes with satisfaction of their sexual desire. Some rats crept successfully, while the rest failed to jump.

Rats of sub-group IIa showed exaggerated aggression during the first 2–3 weeks of the experiment so that their manipulation became difficult. On manipulation, these rats showed ag-

gression in the form of escape, vocalization (crying) and trying to bite the manipulator's hands. Three weeks later, stressed rats showed a gradual decrease in their motor activity to the extent of docility until the end of the experiment. However, other external signs of stress (escape, vocalization and trying to bite the manipulator's hands) persisted until the end of the experiment. However, this aggression caused no death.

Mature male rats of sub-group IIb showed exaggerated aggression in the first week of experiment, which are strung together into sequences that vary in their intensity and duration. The lowest intensity encounters were chases, while stand-offs and physical contacts that might escalate into a fight, such as boxing and sidling, were observed as the intensity increased. This aggression led to the killing of three immature females and contusions and wounds in others. The aggressive behavior of mature males then decreased. Three immature females reached early maturity as revealed by the VO earlier than the expected time of puberty.

Mature female rats of sub-group IIc investigated, sniffed and mounted immature males. They displayed the coital positions and tried to make the mating postures by standing stiff and immobile, arching their backs downwards, elevating their rumps, deflecting their tails to one side and orientating their vulvas backwards. In contrast, immature males showed no copulatory behavior. Although mature females showed an exaggerated aggression towards immature males, it did not lead to death; however, there were contusions and wounds in some immature males. The aggressive behavior of female rats was then decreased. Two immature male rats reached early maturity as revealed by balanopreputial separation earlier than the expected time of puberty.



### 3.2. Vaginal smear cytological findings

Vaginal smears of the control group females revealed normal cycling patterns (Table 1). Each cycle ranged between 4–5 days and consisted of four phases, i.e., proestrous, estrous, metestrous and diestrous phases. In the diestrous phase, many epithelial cells of different sizes and shapes were found in addition to some leucocytes and mucus. In the proestrous phase, a mixture of epithelial cells, leucocytes and some mucus were observed. In the estrous phase, cornified cells were predominating, with a few epithelial cells and leucocytes. In the metestrous phase, leucocytes were predominating, with the presence of a large number of epithelial cells and a considerable amount of mucus. In contrast, stressed female rats showed normal cycling patterns in the first four cycles at the beginning of the experiment (Table 1). After that, estrous cycles were stopped in the diestrous phase in all females throughout the experimental period (Table 2). Vaginal smears showed a large number of epithelial cells of different sizes and shapes, with some leucocytes and mucus.

### 3.3. Hormonal findings

The mean T3 and T4 levels of the male and female experimental groups were significantly lower than those of the control (Table 3), with no statistically significant differences ( $P < 0.05$ ) between all experimental sub-groups. In contrast,

the mean levels of TSH were not significantly affected in the experimental sub-groups compared to the control ( $P < 0.05$ ).

**Table 1.** Cytological patterns of vaginal smears in control and experimental female rats over the first four estrous cycles

Phase (day)	Cytological patterns of vaginal smears			
	Epithelial cells	Cornified cells	Leucocytes	Mucus
<b>Control group</b>				
Proestrous (day 1)	+	+	+	+
Estrous (day 2)	++	++++	+	+
Metestrous (day 3)	+	+	+++	+
Diestrous (day 4)	+++	-	+	+++
Diestrous (day 5)	+++	-	+	+++
<b>Experimental group</b>				
Proestrous (day 1)	+	+	+	+
Estrous (day 2)	++	++++	+	+
Metestrous (day 3)	+	+	+++	+
Diestrous (day 4)	+++	-	+	+++
Diestrous (day 5)	+++	-	+	+++

**Table 2.** Cytological patterns of diestrous phases of the estrous cycles from the fifth cycle until the end of experiment in experimental female rats

Diestrous phase days	Cytological patterns of vaginal smears			
	Epithelial cells	Cornified cells	Leucocytes	Mucus
Day 21	+++	+	+	+++
Day 22	+++	+	+	+++
Day 23	+++	-	+	+++
Day 24	+++	-	+	+++
Day 25	+++	-	+	+++

**Table 3.** Serum thyroid hormones in experimental compared to control rat groups

Group	T3 (mean ± SD)		T4 (mean ± SD)		TSH (mean ± SD)	
	Male	Female	Male	Female	Male	Female
Control	8.68±0.87 <sup>a</sup>	8.78±0.62 <sup>a</sup>	5.81±0.96 <sup>a</sup>	5.28±1.59 <sup>a</sup>	2.11±0.52 <sup>a</sup>	2.09±0.39 <sup>a</sup>
Sub-group IIa	7.87±2.07 <sup>b</sup>	6.58±0.62 <sup>b</sup>	5.16±0.57 <sup>b</sup>	4.82±1.59 <sup>b</sup>	1.92±0.58 <sup>a</sup>	1.86±0.74 <sup>a</sup>
Sub-group IIb	7.27±0.57 <sup>bc</sup>	6.35±0.61 <sup>b</sup>	4.92±0.66 <sup>b</sup>	4.46±1.09 <sup>b</sup>	2.01±0.45 <sup>a</sup>	2.04±0.39 <sup>a</sup>
Sub-group IIc	7.34±0.59 <sup>bc</sup>	6.93±0.61 <sup>b</sup>	4.39±0.83 <sup>bc</sup>	4.23±0.64 <sup>bc</sup>	1.87±0.58 <sup>a</sup>	1.89±0.76 <sup>a</sup>
Statistical significance	*	*	*	*	NS	NS

SD, standard deviation; Means by the same letter were not significantly different at  $P < 0.05$  using Duncan's multiple range test; The symbol "a" refers to the highest mean, while "b" refers to the next lower mean and so on; \*, statistically significant at  $P < 0.05$ ; NS, non-statistically significant at  $P < 0.05$ .



**Table 4.** Serum gonadal hormones in experimental compared to control rat groups

Group	LH (mean ± SD)		FSH (mean ± SD)		Testosterone (mean ± SD)	
	Male	Female	Male	Female	Male	Female
Control	1.99±0.81 <sup>a</sup>	4.59±0.87 <sup>a</sup>	4.57±0.91 <sup>a</sup>	7.46±0.88 <sup>a</sup>	7.57±1.13 <sup>b</sup>	0.82±0.15 <sup>a</sup>
Sub-group IIa	1.15±0.60 <sup>b</sup>	3.48±0.95 <sup>b</sup>	4.45±0.59 <sup>a</sup>	5.82±0.72 <sup>b</sup>	4.91±1.51 <sup>c</sup>	0.74±0.18 <sup>ab</sup>
Sub-group IIb	1.71±0.67 <sup>ab</sup>	3.39±0.96 <sup>b</sup>	3.93±0.64 <sup>a</sup>	5.99±0.68 <sup>b</sup>	10.69±2.14 <sup>a</sup>	0.61±0.16 <sup>b</sup>
Sub-group IIc	0.56±0.84 <sup>bc</sup>	2.03±0.60 <sup>c</sup>	4.32±0.69 <sup>a</sup>	4.06±0.58 <sup>c</sup>	4.88±0.89 <sup>c</sup>	0.76±0.11 <sup>ab</sup>
Statistical significance	**	**	NS	**	**	**

Group	Estradiol (mean ± SD)		Progesterone (mean ± SD)		Prolactin (mean ± SD)	
	Male	Female	Male	Female	Male	Female
Control	1.02±0.21 <sup>a</sup>	8.04±0.74 <sup>c</sup>	6.89±1.02 <sup>a</sup>	23.98±2.85 <sup>a</sup>	4.48±0.66 <sup>b</sup>	1.02±0.21 <sup>a</sup>
Group IIa	0.84±0.21 <sup>ab</sup>	11.50±3.01 <sup>b</sup>	6.31±1.46 <sup>a</sup>	18.75±0.81 <sup>b</sup>	4.91±0.65 <sup>b</sup>	0.84±0.21 <sup>ab</sup>
Group IIb	1.03±0.22 <sup>a</sup>	13.13±1.94 <sup>b</sup>	6.17±1.37 <sup>a</sup>	19.51±1.58 <sup>b</sup>	9.53±1.02 <sup>a</sup>	1.03±0.22 <sup>a</sup>
Group IIc	0.72±0.34 <sup>b</sup>	18.01±2.71 <sup>a</sup>	5.82±1.63 <sup>a</sup>	15.21±1.81 <sup>c</sup>	5.14±1.39 <sup>b</sup>	0.72±0.34 <sup>b</sup>
Statistical significance	NS	**	NS	**	**	NS

SD, standard deviation; Means by the same letter were not significantly different at  $P < 0.05$  using Duncan's multiple range test; The symbol "a" refers to the highest mean, while "b" refers to the next lower mean and so on; \*\*, statistically significant at  $P < 0.01$ ; NS, non-statistically significant at  $P < 0.05$ .

The mean LH Levels of the male and female experimental sub-groups were significantly lower than the control ( $P < 0.05$ ), except for the males of sub-group IIb (Table 4). No statistically significant differences ( $P < 0.05$ ) were observed in the mean levels of LH between all experimental male sub-groups, whereas the mean LH level of the experimental females in sub-group IIc was significantly lower than those of other experimental female rat sub-groups (IIa and IIb) ( $P < 0.05$ ). On the other hand, the mean FSH levels were not significantly affected in all experimental male sub-groups compared to the control, whereas its mean levels were significantly lower in experimental female sub-groups compared to the control ( $P < 0.05$ ). Moreover, the mean FSH level of sub-group IIc was significantly lower than those of other female sub-groups (IIa and IIb) (Table 4).

The mean testosterone level of the experimental male rats of sub-group IIb was significantly higher ( $P < 0.05$ ), while those of the sub-groups IIa and IIc were significantly lower than that of the control ( $P < 0.05$ ). In contrast, it was significantly

lower in experimental female rats of sub-group IIb, with no significant differences compared to other experimental sub-groups (IIa and IIc) (Table 4).

The mean estradiol level was only significantly lower in experimental male rats of sub-group IIc compared to the control ( $P < 0.05$ ). In contrast, it was significantly higher in experimental female rats of all sub-groups, being the highest in sub-group IIc (Table 4). On the other hand, the mean progesterone level was not significantly affected in all experimental male sub-groups compared to the control; however, it was significantly lower in experimental female rats of all sub-groups. Moreover, the mean estradiol level of sub-group IIc was significantly lower than those of other female sub-groups (IIa and IIb) (Table 4).

The mean prolactin level of the experimental male rats of sub-group IIb was significantly higher than those of the control and other experimental sub-groups. On the other hand, the mean prolactin levels of all experimental female sub-groups were significantly higher than that of the



control, being the highest in sub-group IIc (Table 4).

### 3.4. Histopathological findings

#### 3.4.1. Findings in the control group

Thyroid sections of both male and female control rats showed that the thyroid gland consisted of follicles of various sizes (Figure 1). Its walls were lined by cuboidal follicular epithelial cells surrounding colloid inside its lamina. Interfollicular cells were observed between the follicles. In addition, testis sections of the control group revealed normal testicular tissues, no atrophic changes and complete spermatogenesis, with no congestion or polymorphonuclear (PMN) infiltration (Figure 1). Sections of the ovaries revealed normal ovarian tissues with the presence of all stages of folliculogenesis and corpora lutea, with no dysplasia, congestion or PMN infiltration.

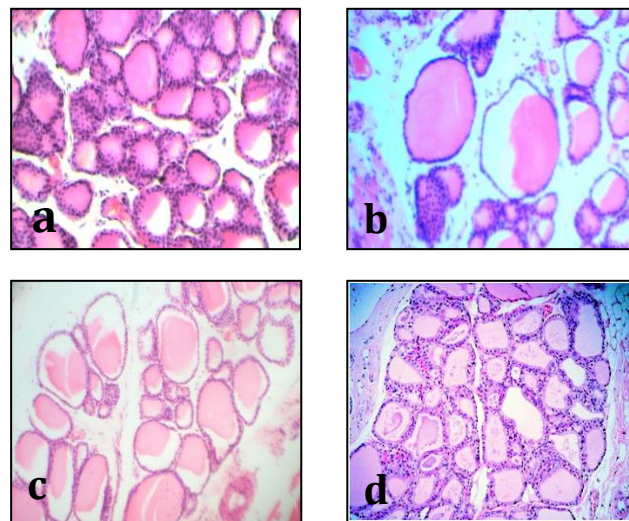
#### 3.4.2. Findings in the males of experimental sub-groups

Thyroid sections showed the enlargement of the thyroid follicles, which were filled with a dense colloid substance and lined by flattened follicular cells, with proliferated mucous glands and increased lymphocytes in the lumen (Figure 1b). Testis sections of sub-group IIa showed partial atrophic changes within the acini, maturation arrest and a decrease in the number of spermatogenic cells in most tubules (Figure 2b). In sub-group IIb, the tubule was fully mature, with numerous sperms and spermatids in the lumen (Figure 2c). In sub-group IIc, the tubules were dilated and enlarged with fully mature sperms, with few sperms and numerous spermatids in the lumen (Figure 2d). In all experimental sub-groups, the stroma showed increased vascularity (congested stroma) and increased PMN infiltration, with minimal hyperplasia of Leydig cells. In

addition, the testicular capsule showed a partial thickening.

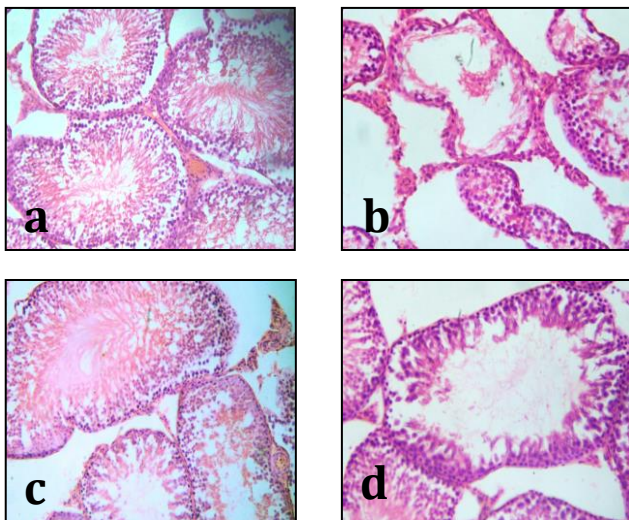
#### 3.4.3. Findings in the females of experimental sub-groups

Thyroid sections revealed increased, proliferated mucous glands and stromal inflammatory cells, mainly neutrophils and eosinophils (Figure 1d). On the other hand, ovary sections showed all stages of folliculogenesis, i.e. primordial, growing and mature Graafian follicles and corpora lutea, with more mature Graafian follicles being detected (Figure 3b). The stroma showed many luteinized cells, PMN infiltration and congestion.

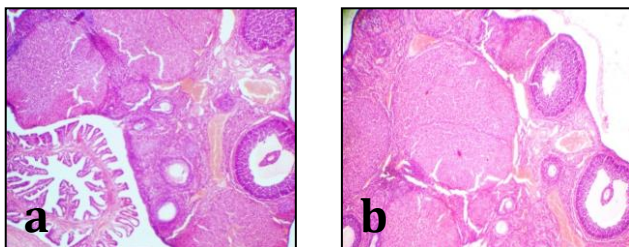


**Figure 1.** Histology sections of thyroid gland: a) Thyroid gland from control male rats; b) Thyroid gland of experimental male rats showing follicles of various sizes; c) Thyroid gland from control female rats with Follicles of various sizes; d) Thyroid gland of experimental female rats showing increased proliferated mucous gland and stromal inflammatory cells mainly neutrophils and eosinophils.





**Figure 2.** Histology sections of testes: a) Testes of control rats showing normal testicular tissues, no atrophic changes and complete spermatogenic process; b) Testes of experimental rats (group IIa) showing partial atrophic changes within the acini, maturation arrest and decrease number of spermatogenic cells in most tubules; c) Testes of experimental rats (group IIb) showing full maturation in tubule and more sperms and spermatid in the lumens; d) Testes of experimental rats (group IIc) showing that tubules were dilated and enlarged with full maturation sperm but less in number in the lumen with more spermatid.



**Figure 3.** Histology sections of ovaries: a) Ovaries of control rats showing normal ovarian tissues with presence of all stages of folliculogenesis and corpora lutea; b) Ovaries of experimental rats show all stages of folliculogenesis (i.e. primordial, growing and mature Graafian follicles in addition to corpora lutea. More Graafian follicles are detected.

#### 4. Discussion

Sexuality is a complex process that is coordinated by the neurological, vascular and endocrine systems. Individually, it incorporates family, social and religious beliefs in addition to being altered with aging, health status and personal experience. Sexual activity is affected by interper-

sonal relationships, where each partner brings unique attitudes, needs and responses to the coupling process. Sexual dysfunction may result from the failure in any of these areas (13). Hormonal imbalance is quite common, particularly in the present time, due to several factors like stress, late marriage, early sexual copulation, life style changes and environmental changes. When hormone levels fluctuate, they cause a lot of mental and physical disturbances in body functions, leading to mood swings and changes in sexual desire (14). Stress response acts as an alarm system that helps the organism to cope with any actual or potential threats to survival or homeostasis (15).

Rats of local strain were used because their reproductive cycles, which last for four to five days, are short enough to detect the pathophysiological changes across many successive cycles within a short duration. In addition, the rat reproductive system and its hormonal control are a good model to study those changes in humans. Although there is no menstruation at the end of reproductive cycles in rats, structural and functional changes in the reproductive organs are closely related to those in humans (16).

In the present work, results revealed that stressed female rats showed normal cycling patterns for the first four cycles of the experiment, followed by cessation of the estrous cycles in all rats. All stressed females, the cycles stopped in the diestrus phase throughout the experiment. This is in agreement with a previous study that reported lengthening of cycles in rats subjected to stress due to prolonged diestrus (7). Stressed rats also showed reduced estrous cycles. Disruption of the estrous cycles as a result of stress could be explained by disturbed hormonal profile during stress, which differs according to different phases of general adaptation syndrome. The organism recognizes a threat (alarm phase) and pushes different body systems into an





arousal state (resistance phase) by activating the HPA and the HPG axes. Finally, if the organism fails to cope with the stress or, its body systems may fail to perform their expected functions (exhaustion phase).

Behavioral observations in the present study showed an exaggerated aggression between experimental rats during the early weeks of the experiment followed by depressed motor activity. An earlier experimental study investigating the effects of acute and chronic restraint stress exposure on the incidence of emotional responses throughout a three-week period among adult rats showed more aggressive behaviors among those exposed to chronic restraint stress (17). Individually, caged animals are susceptible to stress. Rats housed alone usually show pronounced stress-like behavioral changes and altered cardiovascular functions during common husbandry and experimental procedures (18). Moreover, grouping of rats should be done before they reach puberty to avoid or minimize the problems of aggression between unfamiliar individuals (19). In addition, the present study showed that adult males are more aggressive towards immature females in comparison to the aggression of adult females towards immature males. This observation is in agreement with an earlier study reporting that male rats are more likely to be aggressive than females (20). This could be attributed to the fact the dominance hierarchy developed by males, which is less common in females. It has been observed that resident males are more likely to attack intruders in mixed-sex colonies than do the resident females. However, such a low level of female aggression towards intruders may be due to the presence of males (21). It is to be noted that stimulating the brain's aggression mechanism raises blood levels of the stress hormone corticotropin-releasing hormone leading to the aggression (22). Therefore, there is a mutual reinforcement between

stress and aggression imposed by a fast-acting feedback loop.

As regards the sexual behavior, stressed rats tried to overcome barriers that interfere with complete sexual satisfaction as observed by jumping to their partners' compartments, which could be explained by the hierarchy of needs. Sexual behavior is one of the physiological needs that must be satisfied before achieving other needs. Unsatisfied need could lead to regression to a lower level of needs to be satisfied. Differences in the behavioral responses among the experimental groups could be related to the characters of stress and level of maturity. For instance, three immature female rats reached maturity by VO earlier than the expected time of puberty, which usually occurs at 35-42 day (23), and two immature male rats reached maturity by balanopreputial separation earlier than the expected time of puberty, which usually occurs at 39-47 days (24). This result is in agreement with that of a previous study demonstrating that exposure of weaned female house to fresh male mouse urine accelerated puberty as evidenced by VO (25). In addition, anestrus has been observed among female rodents caged in groups, whereas synchronization of estrous cycles and earlier sexual maturation of young female mice have been reported with the presence of a male mouse or its odor (26).

The significant decrease in serum T3 and T4 levels among experimental rats compared to controls in the present study is compatible with an earlier study reporting that stress can cause hypothyroidism through the disruption of the HPA axis, which down-regulates thyroid function (27). In addition, the reduced conversion of T4 to T3 weakens the immune system and promotes autoimmunity, leading to thyroid hormone resistance and hormonal imbalances (28). Increased estrogen levels in the blood caused by chronic cortisol elevations increase the levels of



thyroid-binding globulin, leading to hypothyroidism symptoms by decreasing levels of active T3 (29). In contrast, serum TSH level was not significantly changed compared to the controls, which is in agreement with a study showing unchanged TSH levels with exposure to a new environment or a concentrated ether vapor (30). Furthermore, the observed decrease in thyroid function in the present study was accompanied by histopathological changes in the thyroid.

The significantly lower levels of LH in experimental male rats compared to the controls is in agreement with a previous study that showed significantly decreased LH levels in adult male Wistar rats in response to both acute and chronic stress (31). On the other hand, the significantly lower FSH levels in experimental female rats and unchanged levels in male rats compared to the controls are in agreement with previous study that showed unchanged FSH levels in male rats after acute or chronic stress (31). These findings indicate that stress induces dissociation in LH and FSH responses in male rats. The significantly higher testosterone levels in adult mature rats compared to the controls in the present study could be explained by continuous sexual arousal by immature females. Significant elevation in serum testosterone levels has been reported following prolonged exposure to psychological stress, but decreased hepatic clearance of testosterone could not be ruled out (32). In spite of decreased LH and FSH levels, testosterone was elevated in adult mature rats, which could be explained by catecholamine release during stress that acts peripherally, leading to increased testosterone level as a result of increased blood flow through the testes and/or through direct stimulation of testosterone release by catecholamines (33).

Our results revealed a significant lower level of estradiol in mature rats but a significantly higher level in mature females compared to the

controls. Increased estradiol levels may be an adaptive measure rather than a complication of stress. However, such a protective effect of estradiol against stressful stimuli could not be generalized. Generally, small amounts of estradiol appear to enhance the inhibitory effect of stress on gonadotropin secretion while they exert a protective action against the effect of the inflammatory-like stress challenge in females (34). The present study also showed unchanged progesterone levels among experimental male rats but significantly lower levels in experimental females compared to the controls. In the present study, decreased LH, FSH, testosterone and progesterone and increased estradiol were accompanied by decreased spermatogenesis and decreased number of testis germinal cells in male rats as well as fully mature Graafian follicles, PMN infiltration and congestion of the ovaries in female rats.

In the present study, the applied stress causes disturbed basal prolactin levels among both sexes, where a significantly higher level of prolactin was found among mature males and females compared to the controls. This result is in agreement with previous study reporting increased serum prolactin levels in female rats upon introduction of two stress factors, such as the exposure to a new environment and to concentrated ether vapor (30). During the increase in serum prolactin levels, pituitary prolactin release becomes stress-susceptible. Higher serum prolactin in mature male rats compared to the controls and other experimental sub-groups may be due to the presence of a female besides the male rat, which provoked sustained stimulation of sexual desire.

## 5. Conclusions

In conclusion, the results of the present study highlighted the impact of sexual stress on sexual



behavior and the reproductive-endocrinal related parameters. Sexual excitation is one of the stressful factors that affect sexual behavior, HPT and HPG axes as well as sex organs with secretory functions. Therefore, it is considered as a socio-pathological factor that needs more specific studies to further clarify its effects.

## Authors' contributions

All authors are equally contributed.

## Competing interests

The authors declare that they have no competing interests associated with this article.

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