

## Effects of Immobilization Stress on Some Reproductive Functions in Adult Female Albino Rats

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### Abstract

**Background:** Various factors can disrupt the female reproductive cycle resulting in subfertility. Reproductive disorders and hazards to reproductive health have become prominent public health issue.

**Aim of Work:** Evaluation of the effects of immobilization stress on some reproductive functions in adult female albino rats.

**Material and Methods:** Twenty adult female albino rats of local strain weighing 145-160g were chosen to be the model of the present study. They were divided into two equal groups: Group I (Control group) received no treatment, and Group II (immobilization group) were subjected to immobilization stress for two weeks. Estrus cycle was examined by daily vaginal smear for determination of its phases. Blood samples were withdrawn and serum was separated for determination of FSH, LH, estrogen, progesterone, corticosterone, and prolactin serum levels.

**Results:** Chronic stress was associated with disturbed estrus cycle, gonadotrophic, estrogen, progesterone, corticosterone, and prolactin hormones. Taken together these disturbances affect the female reproductive functions.

**Conclusion:** Chronic stress has a drawback effects on the female reproductive functions. So, a great importance must be considered to avoid stressful situation aiming at avoidance of its drawback effects on the body functions.

**Key Words:** Stress – Immobilization – Reproduction – Hormones.

### Introduction

**PHYSIOLOGICAL** systems act within a coordinated manner to maintain stability of internal, acid-base and electrolyte balance. Chronic stress lead to activation of neuroendocrine system that promote adaptation response [1]. Chronic immobilization stress can lead to many negative health problems,

including physical and emotional problems. The female fertility can be deteriorated by various types of stressors [3].

When the body is stressed, the neuro-endocrinal system are stimulated and the process begin to produce catecholamine's and cortisol. If we are in a state of prolonged stress, high levels of these hormones suppress the female gonads and this will lead to loss of libido and reproductive function [4].

The glucocorticoid works on the hypothalamic-pituitary-gonadal (HPG) axis to induce infertility. These effect through the hypothalamus and portal circulation levels to reduce the secretion of the gonadotrophin and the pituitary level to reduce sensitivity of the gonadal cells to the release of gonadotropin [5].

The production of B-endorphin peptide induced by chronic stress reduce the production of gonadotropin-releasing hormone (GnRH) resulting a decrease release of LH and thereafter leads to loss of ovulation. Also, chronic stress produce poor growth of ovarian follicles, this ovarian dysfunction refer to suppression GnRH-LH secretion which protect ovarian follicle from gonadotropin effect leading to inhibition of estrogen and progesterone [6].

Elevated level of glucocorticoids as a result of stress leading to free radical formation and accordingly decrease synthesis of female sex hormones. Also, estrogen deficiency with chronic stress may lead to production of reactive oxygen species and thereafter depress oocyte maturation [7].

The present work was designed to evaluate the effects of immobilization stress on reproductive functions in adult female albino rats.

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### Patients and Methods

The experimental protocol and animal handling were approved and performed according to the guidelines of animal use of the Ethical committee of Faculty of Medicine, Al-Azhar University. The work was done from Nov. 2017 to Feb.2018. Twenty adult female albino rats of local strain weighing 145-160g were chosen to be the model of the present study. They were left for two weeks in the laboratory room before any experimental interference for acclimation with free access to water and rat chow pellets. Rats were kept in suitable cages (40 x 30 x 30 per 5 rats) at room temperature with the natural light-dark cycle. Rats were divided into two equal groups:

- Group I (Control group) received no treatment.
- Group II (Immobilized group) were subjected to induction of immobilization stress for 6 hours/day for two weeks by placing them into transparent plastic tubes of a size sufficient to induce stress without promoting unnecessary pain. The tube was 6cm inner diameter, having a 4cm long conical head part ending with a large breathing hole [4].

Estrus cycle was examined by daily vaginal smears for determination of its phase. Using a suitable dropper, a drop of saline was put into the vagina of each female, then was withdrawn again. The contents were spread over a glass slide and examined using a light microscope to determine the different cell types. Cell types determined included cornified epithelial cell, epithelial cells, white blood cells and mucus. Predominant cells were plotted in a specially made chart. The proportion among them was used to determine the phase of estrus cycle [8].

At the end of the experimental period, blood samples were withdrawn from the retro-orbital plexus into test tubes. Sera were separated and stored frozen at  $-20^{\circ}\text{C}$  until assayed for determination of the levels of serum follicle stimulating hormone [9], luteinizing hormone [10], estrogen [11], progesterone [12], corticosterone [13], and prolactin [14].

*Statistical analysis:* Data analysis were done using SPSS computer program. All results were expressed as mean  $\pm$  standard error. Mean values of the different groups were compared using a one-way analysis of variance (ANOVA). Least significant difference (LSD) post hoc analysis was used to identify significantly different mean values. *p*-value  $<0.05$  was accepted to denote a significant difference.

### Results

Rats of the control group displayed normal cycling pattern. Each cycle ranged between four to five days and consisted of four phases, i.e. proestrus, estrus, metestrus and diestrus phases. In the proestrus phase, a mixture of epithelial cells, leucocytes and some mucus were observed. In the estrus phase, cornified cells were predominating. However, few epithelial cells and neglected leucocytes were also found. In the metestrus phase, leucocytes were predominating with the presence of a considerable amount of epithelial cells and mucus. In the diestrus phase, many epithelial cells of different sizes and shapes were found in addition to some leucocytes and mucus (Table 1).

In contrast to the control group, stressed female rats showed arrested cycle in the diestrus phase throughout the experiment length. Vaginal smear showed mainly epithelial cells of different sizes and shapes in addition to some leucocytes and mucus (Table 2).

Results of the present work showed that induction of immobilization led to significant increase in serum FSH level from  $0.41 \pm 0.03\text{IU/ml}$  to  $0.58 \pm 0.04\text{IU/ml}$  (+41.46%), significant decrease in serum LH level from  $0.76 \pm 0.08\text{IU/ml}$  to  $0.51 \pm 0.04\text{IU/ml}$  (-32.89 %), significant decrease in serum estrogen level from  $22.95 \pm 1.21\text{Pg/ml}$  to  $12.89 \pm 1.32\text{Pg/ml}$  (-43.83%), significant decrease in serum progesterone level from  $18.87 \pm 1.23\text{ng/ml}$  to  $7.81 \pm 0.92\text{ng/ml}$  (-58.61%), significant increase in serum prolactin level from  $2.2 \pm 0.7\text{ng/ml}$  to  $4.9 \pm 0.6\text{ng/ml}$  (+31.81 %), and significant increase in serum corticosterone level from  $71.8 \pm 4.12\text{ng/ml}$  to  $98.9 \pm 6.14\text{ng/ml}$  (+37.74% - Table 3).

Table (1): Pattern of Estrus cycle in the control group.

Parameters Days	Epithelial cells	Cornified cells	Leucocytes	Mucus	Phase
1st day	+	+	+	+	Proestrus
2nd day	++	++++	+	+	Estrus
3rd day	+	+	+++	+	Metestrus
4th day	+++	-	+	+++	Diestrus
5th day	+++	-	+	+++	Diestrus

"*p*-value of  $<0.05$  was considered as significant".

Table (2): Pattern of Estrus cycle in the experimental group.

Parameters Days	Epithelial cells	Cornified cells	Leucocytes	Mucus	Phase
1st day	+++	+	+	+++	Diestrus
2nd day	+++	+	+	+++	Diestrus
3rd day	+++	-	+	+++	Diestrus
4th day	+++	-	+	+++	Diestrus
5th day	+++	-	+	+++	Diestrus

"p-value of <0.05 was considered as significant".

Table (3): Changes of the measured parameters in the tested groups (Mean ± SE).

Parameters	Groups I (n=10)	Groups II (n=10)	% Changes
FSH (IU/ml)	0.41±0.03	0.58±0.04*	+41.46
LH(IU/ml)	0.76±0.08	0.51±0.04*	-32.89
Estrogen (Pg/ml)	22.95±1.21	12.89±1.32*	-43.83
Progesterone (ng/ml)	18.87±1.23	7.81±0.92*	-58.61
Prolactin (ng/ml)	2.2±0.7	4.9±0.6*	+31.81
Corticosterone (ng/ml)	71.8±4.12	98.9±6.14*	+37.74

"p-value of <0.05 was considered as significant".  
n: No. of rats in each group. \*: Significant.

Group I: Control. Group II: Immobilized group.

### Discussion

In the present work, immobilization stress of rats was induced for 6 hours daily for two weeks which was acceptable to develop symptoms of chronic stress as reported by Zsuzsanna et al., [15] who reported that chronic stress is a combined psychological-physical stress stimulus, and seven days of daily stress is sufficient for the animals to produce symptoms of restraint stress.

It has been reported that on exposure to repeated stress, the female's estrus cycle may be troubled which probably denotes different effects on female sexual activity [3].

Stress-induced reproductive impairment is more likely attributed to repeated stress rather than acute stress. Chronic stress is one of the most important challenges in livestock production, as it can lead to immune system depression, increased disease vulnerability and reproductive impairment [16].

Results of the present work showed that rats of the control group displayed normal cycling pattern, while stressed female rats showed arrested cycle in the diestrus phase throughout the experiment length. These results were in agreement with Shamolina et al., [17] who reported that, stress response changes in the non-receptive phase of the cycle (diestrus) compared to proestrus and estrus phase (receptive phases). Also, Nosenko et al., [18] has reported Estrus cycle disturbances in stressed

female rats associated with sudden unexpected decrease or absence of luteal bodies, cysts formation and overgrowth ovarian interstitial tissue.

It has also been reported that the individual duration of the estrus cycle was evaluated using vaginal smears showed that non-stressed female rats revealed regular 5-day cycles. Comparatively, stressed female rats exhibited a significant increase of irregular cycles by about 28% of the examined rats [19].

Results of the present work showed that induction of immobilization led to increased serum FSH and LH levels. These results were compatible with Traslaviña and Franci [20] who reported that chronic stress increased the levels of FSH and LH, and postulated that impairment of reproductive function by chronic stress is mediated by corticotrophin releasing hormone (CRH) action. The (CRH) perform via corticotrophin releasing hormone receptor 1 on noradrenaline neurons residing in the locus ceruleus that excite gonadotropin releasing hormone (GnRH) production and gonadotropin release (FSH and LH). Traslaviña and Franci [21] has also reported that high plasma level of LH produced by restraint stressful situation was followed by disturbed secretion manner in the estrogen-induced surge. Fotsing et al., [22] has also reported that repeated immobilization stress (3 hours per day) was used to induce female reproductive and behavioral disturbances in rats, and concluded that restraint rats showed significantly disturbed estrus cycle phases

combined with significantly enhanced follicle stimulating hormone and luteinizing hormone secretion compared to the control.

It has been reported that repeated stress leads to modification in gonadotropin release from the anterior pituitary, possibly by affecting gonadotropin releasing hormone (GnRH) from the hypothalamus. Immobilization stress utilized on the day of proestrus phase has been reported to suppress luteinizing hormone (LH) surge and ovulation in rats with regular cycle [23].

Results of the present work showed that induction of immobilization led to significant decrease in both estrogen and progesterone serum level. These results were in agreement with Liang et al., [24] who reported that chronic stress suppress the production of estrogen hormone, debilitate oocyte competence causing a latent apoptotic process in the cumulus cells and oocytes during their intraovarian growth.

It has also been reported that suppression of the hypothalamo-pituitary-gonadal axis in response to stress leads to altered secretion of GnRH, FSH, LH, progesterone and estradiol, in addition to drawback effects on the reproductive functions [25,26].

Fotsing et al., [22] has also reported that repeated immobilization led to significantly reduced estradiol, FSH and LH compared to stressed rats treated with *Gladiolus dalenii* plant extract, and concluded that *Gladiolus dalenii* plant extract antagonizes the action of stress-induced reproductive, neurochemical and behavioral disturbances.

Enhanced hypothalamo-pituitary adrenal (HPA) axis activity due to chronic stress can lead to reproductive impairment by modify molecular and cellular functions of the HPG axis. The effectiveness of HPA-HPG axes interact on the reproductive function and can disturb fertility, rate and ovulation quality and increase embryonic and offspring mortality [6].

Results of the present work showed that induction of immobilization led to significant elevation in serum prolactin level. This result was in correspondence with Zografos et al., [27] who reported that the response to stressful situation involves the liberation of growth hormone and prolactin. Also, the high level of growth hormone, cortisol and catecholamines, as a part of the stress response, result in elevated blood glucose level.

Prolactin hormone levels are known to be impaired by psychological stress. Chronic psychological stress leads to significantly elevated serum prolactin levels [28]. Elevated serum prolactin hormonal levels can also disrupt sexual activity. The degree of the prolactin response could be related to the extent of the HPA axis response to stressful situations [29].

Fotsing et al., [22] has also reported that repeated stressed female rats showed significantly elevated serum prolactin level compared with stressed rats treated with *Gladiolus dalenii* plant extract.

Results of the present work showed that induction of immobilization stress led to significant elevated serum corticosterone level. This result was in correspondence with Nosenko et al., [30] who reported that stressed females rats showed significantly elevated corticosterone levels. In addition, the degree of adrenals activation is increased gradually in stressed rats but the reaction to stress was suppressed in androgenized 45-day old females.

Tamburella et al., [31] reported that elevated corticosterone serum level associated with chronic repeated stress refers to neuroendocrine responses, and point that stress perform this action by a disturbances in HPA axis and mechanisms responsible for neuronal plasticity. Gong et al., [32] has reported that unpredictable stress led to elevated cortisol and corticosterone hormones to the highest level on the first day of stress. After that, the concentration of cortisol remain unchanged, while that of corticosterone showed fluctuate level. Thus, while corticosteroids decreased significantly during chronic restraints, they remained at a high level during an unexpected stressful state.

Elevated corticosterone levels could be demonstrated by stimulation of the HPA axis. The response to stress initiated by activation of the parvocellular neurons of the paraventricular nucleus in the hypothalamus. These neurons liberates CRH into the hypophyseal portal vein, which stimulates the production of ACTH from the anterior pituitary, and finally production of corticosterone from the adrenal gland into the blood [33].

Activation of the HPA axis is associated with suppression of the HPG axis by glucocorticoids through inhibition of GnRH at the hypothalamic level. The main regulators of the HPA axis include CRH, glucocorticoids and ACTH [34].

*Conclusion:* Chronic repeated stress has great drawback effects on the female reproductive func-

tions. So, it is of great importance paying attention for avoidance of stressful situations to avoid its obstacle effects on the body functions and improving quality of life.

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## تأثير ضغط تحديد الحركة على بعض وبعض وظائف التكاثر في إناث الجرزان البيضاء

خلفية البحث: العديد من العوامل تؤثر وتؤدي إلى اضطراب دورة التوالد للأنثى مما يؤدي إلى ضعف الخصوبة. وقد أصبحت اضطرابات نقص الخصوبة والتوالد من المشاكل الصحية العامة المتصدرة للبحوث.

الهدف من البحث: تقييم تأثير ضغط تحديد الحركة على بعض وبعض وظائف التكاثر في إناث الجرزان البيضاء البالغة.

مواد وطرق البحث: استخدم في هذا العمل عشرون أنثى من السلالة المحلية من إناث الجرزان لبيضاء البالغة وقد تم تقسيمهن إلى مجموعتين متساويتين: المجموعة الأولى (مجموعة ضابطة) والمجموعة الثانية (مجموعة الإختبار) تعرضت لضغط تحديد الحركة لمدة أسبوعين.

وقد تم فحص الدورة التناسلية يومياً عن طريق مسحة مهبلية لتحديد طورها. وفي نهاية العمل تم سحب عينات دم وفصل المصل لقياس مستويات الهرمونات المنشطة للمبايض، وهرموني الإستروجين والبروجيسترون وهرمون الكورتيكوستيرون وهرمون البرولاكتين.

الإستنتاج: التعرض لضغط تحديد الحركة يؤثر بصورة مباشرة على الدورة التناسلية للأنثى، بالإضافة إلى اضطرابات هرمونية مما يؤثر على وظائف التكاثر في الإناث. وعلى هذا فمن الأهمية تجنب التعرض لضغط تحديد الحركة لتجنب آثاره السلبية على وظائف الجسم.