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Effects of Pregestational Chronic Restraint Stress on the Physiological State in Female Wistar Rats

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Abstract: Stress is a very important current issues as well as at the human and the animal. Each individual is confronted in his everyday life to stressful situations. Stress activates the hypothalamic-pituitary-adrenal axis (HPA), almost each component of this activated axis exerts profound inhibitory effects on the hypothalamic-pituitary-ovarian axis (HPG). In the present investigation, the application of chronic restraint stress of (1h/ day/4 day/ week/5 weeks) before gestation leads to a reproductive failure in females, perturbation of ovarian steroidgenesis; when we recorded a highly significant increase of the concentration of plasma progesterone, and delayed parturition.

Key words: Chronic Restraint Stress · Female Wistar Rats · Progesterone · Reproductive Parameters

INTRODUCTION

The hypothalamic-pituitary-adrenal axis (HPA) is a key endocrine adaptor against stressors and plays an important role in the pathophysiology of stress-related psychiatric diseases [1]. The interaction between environmental and genetic factors may participate in the installation pathology by disruption of homeostasis. The hypothalamic-pituitary-adrenal (HPA) responses increase after chronic or repeated stress despite robust levels of circulating glucocorticoids [2]. The direct neural connection between CRH and GnRH [3]. The presence of the receptors of Glucocorticoids in the hypothalamic GnRH neurons [4], also in the pituitary gonadotrophs [5]. The Chronic hyperactivation of any components of the HPA axis results behavioural changes such as; decrease in sexual and aggressive behaviour [6]. And is also accompanied with disturbances of the oestrus cycle [7].

Chronic stress conditions like restraint, strenuous exercise, malnutrition, infection and surgical trauma can affect a reproductive functions [8] and causes anovulation due to the suppression of gonadotrophic hormones [9]. Exposure to chronic stress has been shown to be associated with elevated serum progesterone concentration, with depressed gonadotropin release and luteinizing hormone surge LH [10-13]. Other investigators previously have observed that stress caused by forced swimming test can increase progesterone as well as corticosterone secretion [14].

The aim of this study was to see the effects of the psychological stress applied before gestation on the release of progesterone and the alteration of the parameters of reproduction in female Wistar rats.

MATERIALS AND METHODS

Animals: Female albino rats coming from Pasteur Institute of Algiers were used during this study. The rats were acclimatized to the Standard conditions of natural photoperiod, an average temperature of 22 ± 4°C and a relative humidity of 50-70%. After one period of three weeks adaptation, we selected 25 females according to the weight between (140-170) grams then we divided them into 2 lots experimental: a control batch of 17 rats and stressed batch of 17 rats (after stress only 14 rats were used for coupling).

Induction of Stress: Our model of stress is based on that of Bardin [15], at the end of the pallet of the stress a behavioural evaluation was carried out.

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Measurement of Plasma Progesterone: The taking away is done starting from the lachrymal vein at the end of the application of chronic restraint stress. The blood samples are collected in the heparinized tubes then centrifuged at 1500 rpm for 10 minutes. Plasma was used for progesterone assay. The progesterone is proportioned by the conventional ELISA method [16].

Measurement is done using a reader ELISA TECAN Magellan provided with a data-processing software which calculates the range standard automatically and the value of the progesterone to the desired unit gives us directly.

Reproductive Parameters: Parameters of reproduction in the rats of the two batches were evaluated measuring; fertility rate, fecundity rate, rate of numerical productivity, growing mortality, stillbirth rate, Sex ratio.

Data Analysis

Progesterone: The results are presented as mean ± standard error (SEM) and were analyzed by using test T of Student with the program Minitab (version13). They are regarded as being significant $p<0.05$. And for reproductive parameters, we used the chi-square test on Excel office to compare the values less than 100% and the Student test for the values above 100%. *P* values less than 0.05 considered significant.

RESULTS

Variation of Plasma Concentration of Progesterone (ng/ml): The results obtained show that the average of progesterone of stressed rats is higher (74.83± 9.16ng/ml) than the control rats (46.09 ± 7ng/ml).

The statistical analysis of these results revealed a highly significant increase(** $p< 0.01$) of progesterone in stressed rats compared to the control rats (Fig. 1).

Variation of Reproductive Parameters (%): The results obtained show a reduction in the fertility rate (50 %), fecundity (485.714%) and of numerical productivity (435.714%) at the stressed rats compared to the fertility rate (100%), fecundity (550%) and of numerical productivity(550%) of the control rats.

The statistical analysis shows a highly significant reduction in the fertility rate(** $p< 0.01$), a very significant reduction of fecundity and numerical productivity rate (*** $p< 0.01$) at the stressed rats compared to the control rats (Fig.2).

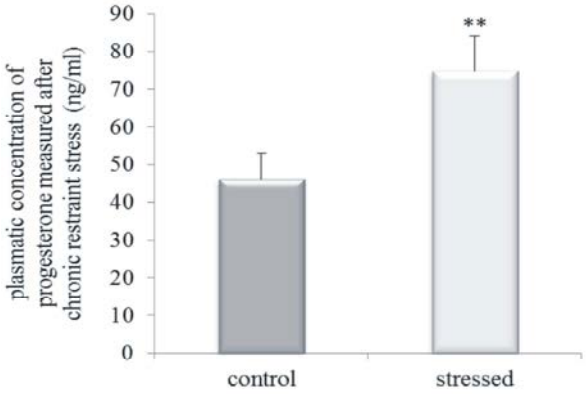

Fig. 1: Variation of plasma concentration of progesterone (ng/ml). (m ± SD; n control=17, n stressed=17). (Ns: $p> 0.05$; *$p<0.05$; **$p< 0.01$; ***$p< 0.001$).

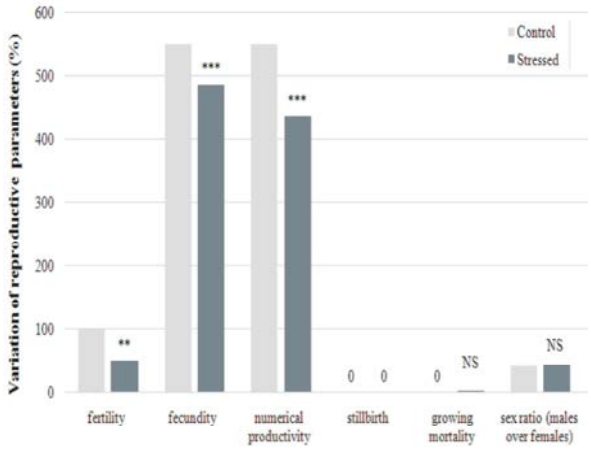

Fig. 2: Variation of reproductive parameters (%). (m ± SD; n control=14, n stressed=14). (Ns: $p> 0.05$; *$p<0.05$; **$p< 0.01$; ***$p< 0.001$).

DISCUSSION

Numerous studies have been carried out to understand the role of various lifestyle factors contributing to stress and the development of pathophysiology. Studies in ovariectomized animals have indicated that either psychological stress or an increment of plasma cortisol level reduces LH pulse amplitude [17, 18], this reduction contribute indirectly to the elevation of progesterone, which have been associated with depressed of gonadotrophin release [12] and luteinizing hormone surge [13]. Our results show that stress induced an elevation in plasma concentration of progesterone which can be mediated solely by adrenal gland, the same result was mentioned by Romeo *et al*. [19].

What is explained by the interaction between ovarian and adrenal steroids to control the secretion of gonadotropin [20], ACTH, corticosterone [21] and adrenal progesterone [22].

Results of Guillermo *et al*. [23] showed after application of restraint stress an elevation in the level of progesterone. Because proopiomelanocortinis precursor synthesis of GnRH and CRH, under stress, proopiomelanocortin is used in large quantities for the synthesis of ACTH, which leads to a decrease of synthesis of GnRHso itsinhibition [24], which result a decrease in pulsatile release of LH [25]. This process is independent of the stress-induced cortisol level [26]. However, prolonged enhanced secretion of cortisol contributes to the suppression of GnRH pulse frequency, but only in the presence of ovarian steroids [27]. Moreover, may negatively affect the reproductive function via actions at the hypothalamus (GnRH) as well as impairing LH release induced by GnRH [28]. Although progesterone has been considered only as a female reproductive hormone, elevated levels of plasma progesterone accompany the increase in corticosterone after stress in male rats [14, 29] and male humans [30]. Stress-induced progesterone secretion in male and female rats is derived from the adrenal gland, because the response is abolished after adrenalectomy [14,29].

Receptors for CRH are identified in most of the female reproductive tissues including the ovary, uterus and placental trophoblast [31]. There is abundant evidence that the gonads affect the way that the HPA axis responds to stress [32]. Van Lier *et al.* [33] evaluated the presence of estrogens receptors in sheep adrenal glands. Ovarian steroids have been found to increase HPA-axis activity, enhance the HPA-axis response to psychological stress and sensitize the HPG-axis to stress-induced inhibition in human and rhesus monkey [34]. Which explains the results obtained on reproductive parameters.

CONCLUSION

The hyper activation of the HPA axis cause an increase of the circulating plasma progesterone, of glucocorticoïds more other hormones can affect various neuroendocrine systems, like the inhibition of the HPG axis who can possibly act as the major cause of impaired fecundity in women.

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