Journal of Applied Biomedicine 16 (2018) 370–377

Contents lists available at ScienceDirect

Journal of Applied Biomedicine

journal homepage: www.elsevier.com/locate/jab

Original research article

Pre-pregnancy stress suppressed the reproductive systems in parents and changed sex ratio in offspring

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ARTICLE INFO

Article history:
Received 18 February 2018
Received in revised form 23 July 2018
Accepted 20 August 2018
Available online 27 August 2018

Keywords:
Predatory stress
Pre-gestation
Reproductive functions
Sex hormones

ABSTRACT

This study aimed to investigate the effect of stress during spermatogenesis and oogenesis on reproductive performance in adult rats and sex ratio in offspring. The rats were subjected to predatory stress (exposed to a cat) twice a day for 50 (male) and 15 (female) consecutive days. At the end of the stress procedure, a number of control and stressed rats were considered to examine reproductive parameters and the rest was coupled as follows: both male and female control, male stressed/female control, male control/female stressed, and both male and female stressed. After parturition, the pups were counted, weighed, and gendered. Stress significantly increased the number of female pups in each litter ($P = 0.034$). In parents, stress reduced sperm quality (mobility, number, and morphology), testicular parameters (SI, STET, sloughing, and detachment), and thickness of vaginal epithelium in all phases of the estrous cycle. Serum testosterone and 17-B estradiol levels decreased significantly in stressed parents. These results emphasize the suppressive influence of stress during spermatogenesis and oogenesis on the performance of the organs of the reproductive system in parents and its consequence on sex ratio in offspring.

Introduction

In many communities, the social and family issue of reproduction is very important. It has been assumed that life stress modifies the dynamic regulation of neuroendocrine and immune systems (Collodel et al., 2008). Among the most regularly hostile consequences of prolonged stress is the disturbance of reproductive physiology and behavior (Wingfield and Sapolsky, 2003). Systems activated by stress can influence reproduction at different levels, including the hypothalamus, pituitary, and gonads. Nevertheless, the main impact is supposed to be within the brain or in the pituitary gland (Tilbrook et al., 2000). There is considerable evidence that corticosteroids, intervening at the level of hypothalamus, pituitary gland, and gonads, mediate the suppressive effects of stress on reproduction (Campbell et al., 1992). Since stress effects are sex-specific (Saboory et al., 2015; Sadaghiani and Saboory, 2010) and these differences are due, at least in part, to the actions of sex hormones, the mechanisms by which stress influences reproduction may also vary between the sexes and be influenced by the predominance of the secretion of certain hormones at certain times (Tilbrook et al., 2000). The suppressive impacts of stress on the reproductive system of female mammals usually have three broad elements: a) interference of ovulation, b) disturbance of the uterine maturation required for implantation, and c) inhibition of proceptive and receptive behaviors (Wingfield and Sapolsky, 2003). In male mammals, the suppressive effects of stress on reproductive functions are mediated in three broad ways: a) inhibition of hormones of the gonadal axis, b) impairment of erection, and c) attenuation of proceptive and receptive behaviors (Wingfield and Sapolsky, 2003). Moreover, it has been reported that stress leads to severe disturbances in the maturation of sperms in testicles and affects all populations of cells in the spermatogenic epithelium. The observed changes result in reproductive dysfunction in male rats (Potemina, 2008). Similarly, it was reported that heat stress in wild-type females results in delays of oocyte maturation, degradation of early vitellogenic egg chambers, inhibition of yolk protein gene expression in follicle

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https://doi.org/10.1016/j.jab.2018.08.002
1214-021X/

cells, and accumulation of mature oocytes (Gruntenko et al., 2003). The ratio of female to male live births reportedly rises when populations suffer infrequent and extreme ambient stressors (Catalano et al., 2005). Since most recent studies on prenatal stress have focused on the impact of stress during the gestational period, the present study attempted to assess the impact of pre-gestational stress during spermatogenesis and oogenesis on reproductive systems in parents and sex ratio in offspring. In addition, most previous studies focused on the effect of stress in one parent (particularly maternal stress) on reproductive physiology and sex ratio. This study is unique because both parents were stressed prior to coupling. Therefore, the present study aimed to investigate the effect of pre-pregnancy stress in both parents on reproductive systems and sex ratio in offspring.

Materials and methods

Animals

The Regional Medical Ethics Committee in West Azarbaijan Province, I. R. Iran, approved this study. All the experimental protocols and procedures complied with the guidelines of the 1975 Declaration of Helsinki as reflected in the guidelines of the Medical Ethics Committee, Ministry of Health I. R. Iran. Male and female Wistar rats (200–250 g) were obtained from the animal facility at Urmia University of Medical Sciences, Urmia, Iran. They were 12 weeks old on delivery. The rats were housed in groups of four per cage and kept in standard conditions as follows: standard 12 h light/dark cycle (light on at 7 a.m.), environmental temperature of $22 \pm 2\,^{\circ}C$, and food and water available *ad libitum*. Effort was made to minimize the number of rats used and their suffering. After 7 days of adapting to the new conditions of the living room, male and female rats were randomly divided into four groups to form a combination of control and stressed groups for each sex ($n = 32$ male and $n = 64$ female). Trichus method was applied for coupling (1 male and 2 female). Four groups of male ($4 \times 8 = 32$) and four groups of female ($4 \times 16 = 64$) used for coupling to complete all possible combination of control and stressed rats (for more details see below). After coupling, the male rats were subjected to evaluation of reproductive system and sex hormone levels; while, another 20 female rats (control and stressed, $n = 10$ each) were used for decapitation and subsequent hormonal and vaginal evaluation.

The stressed rats were exposed to a predatory stressor for 50 consecutive days for male and 15 consecutive days for female rats. Seven weeks (50 days) is the time needed for a complete spermatogenesis cycle in male rats, while 15 days comprise three cycles of estrous cycle in female rats.

Predatory stress procedure

A healthy adult feral cat caught from the Faculty of Veterinary Medicine at Urmia University was used as the predator for rats. The cat was then placed in a stainless steel holding cage ($72 \times 72 \times 63$ cm) consisting of a solid metal floor with a hinged metal rod door and air holes on the side so that the cat could be observed. To induce predator stress, rats were first placed in boxes ($20 \times 22 \times 22$ cm) with multiple air holes in the side walls. The boxes prevented any physical contact between the cat and rats but exposed the rats to all other sensory stimuli such as the sight, smell, and sounds produced by the cat (Ahmadzadeh et al., 2011; Wilson et al., 2014). The rats were predatory-stressed twice a day (between 8–9 a.m. and 4–5 p.m.) for 50 (male) or 15 (female) consecutive days. After each stress session, the rats returned to their home cage. The control rats were transported to another experimental room (other than the cat room) and handled similarly to the stressed rats but were not exposed to stress.

At the end of the stress procedure, the rats were coupled for 72 h using the Trichus method (two females and one male in each cage; 16 females and 8 males in each group) as follows: both male and female control (Mc-Fc), male stressed + female control (Ms-Fc), male control + female stressed (Mc-Fs), and both male and female stressed (Ms-Fs). After identifying the vaginal plug, pregnant rats were immediately moved to new cages in which they were housed three per cage for the entire pregnancy period and kept in normal conditions as previously explained. After parturition, each dam and her pups were moved to a new cage and the pups were counted, weighed, and gendered. The pups were also divided into four groups based on their parents' grouping as follows: Mc-Fc, both parents were control; Mc-Fs, only the female parent was stressed; Ms-Fc, only the male parent was stressed; and Ms-Fs, both parents were stressed.

Blood sample collection

In the morning at 8 a.m. and 11 a.m., the blood samples of control and stressed rats and 30-day-old pups from all the groups were collected by direct heart puncture under anesthesia with isoflurane. Blood was collected in 1.5 ml EDTA-coated micro-centrifuge tubes, kept on ice, and later centrifuged for 15 min at 9000 rpm at $4\,^{\circ}C$. The blood plasma was stored frozen at $-80\,^{\circ}C$ until the determination of testosterone or 17-beta estradiol levels. These hormones were measured using a commercial ELISA kit (Cayman, Ann Arbor, MI, USA, for Estradiol; and Elabscience, Technology Industry Park, Wuhan, Hubei Province, China, for testosterone) and the values were expressed in pg/ml (17-beta estradiol) and ng/ml (testosterone).

Sperm sampling

Stressed and control male rats ($n = 8$ per group) were anesthetized with isoflurane in the morning after the last stress session. The testes were preserved in 10% formalin. Then, both epididymides (caudas) of each rat were transferred to a conical glass Petri dish containing DMEM/F12 (DMEM/F12; ATCDHF, St. Louis, USA) culture supplemented with 20% fetal bovine serum (FBS; Sigma, St. Louis, USA) medium pre-warmed to $37\,^{\circ}C$ (Ornoy et al., 2003). The caudate was minced, making 5–7 slashes with a 30-gauge needle of an insulin syringe. After 30 min of incubation at $37\,^{\circ}C$ in 5% CO_2, spermatozoa were released from epididymides.

Assessment of sperm count

After preparing a 1:20 dilution of spermatozoa using distilled water, 10 μl of the solution was transferred on to a Neubauer slide on which a stone cover slip had been placed before, and then spermatozoa were counted. Also, sperm motility was assessed using a light microscope at $10\times$ and $20\times$ magnifications (Bakhtiary et al., 2015).

Sperm morphology

One drop of the semen was mixed with 2 drops of 1% eosin Y. After 30 s, 3 drops of 10% nigrosin solution were added and mixed. A drop of the semen-eosin-nigrosin mixture was placed on a clear microscope slide, allowed to dry, and observed under the microscope. The sperm morphology of treated sperm was studied under the microscope. A drop of semen was examined separately at $400\times$ under a phase-contrast microscope to record any changes in morphology of the sperm (Khillare and Shrivastav, 2003).

Table 1
Different parameters of sperm in control and stressed rats.

Parameters Movement categories (%):	Control Mean ± SE	Stressed Mean ± SE	*P*
Progressive (A)	74.20 ± 6.88	17.91 ± 5.71	<0.001
Non-progressive (B)	13.86 ± 2.86	31.52 ± 3.92	0.002
No motility (C)	11.80 ± 2.86	50.55 ± 3.92	0.001
Sperm content ($\times 10^6$ Ml)	156.90 ± 11.16	105.50 ± 10.88	0.005
Morphology (% normal)	64.18 ± 3.86	34.44 ± 1.62	<0.001

Histopathology of testes

The testes were fixed in 10% formalin and embedded in paraffin. Then, 5 μm-thick sections were prepared and stained with hematoxylin and eosin (H&E). Afterwards, the diameter (μm) of the seminiferous tubule (DST), spermatogenesis index (SI), seminiferous tubule epithelium thickness (STET) (μm), detachment (appearance of breaking off of cohorts of spermatocytes from the seminiferous epithelium), and sloughing (release of clusters of germ cells into the lumen of the seminiferous tubule) in the sections were calculated. The diameters of 25 seminiferous tubules and the thickness of seminiferous tubule epithelium in each rat were measured with the Motic Images software. To evaluate SI, 200 seminiferous tubules in one section from each rat were scored for sperms present in each tubule. A total of 150–200 seminiferous tubules per rat were examined for signs of germ cell degeneration including the following histopathological alterations: detachment and sloughing.

Vagina sampling

At the end of the stress, pap smears were prepared from the female rats to determine the estrous cycle phase (proestrus, estrus, metestrus and diestrus) of each rat. Vaginal secretions were collected with a cotton swab moistened with normal saline by inserting the tip into the rat vagina, but not deeply. Vaginal fluid was placed on glass slides. Unstained material was observed under a light microscope, without the use of condenser lens, with 10× and 40× objective lenses (Hou et al., 2014). At least 2 h from pap smear, vaginal pH was measured using pH meter paper (Forozanfard et al., 2014). The paper was inserted into the rat vagina up to 1/3 of vaginal length with forceps, held inside the vagina for 5 min, and compared and read with a pH meter scale for any color changes. Finally, under anesthesia with isoflurane, vaginal samples were obtained. All tissues were fixed in 10% neutral buffered formalin, embedded, sectioned, and stained with H&E using routine procedures. Vaginal epithelial thickness was measured using Motic Images. The software was calibrated for each objective using a stage micrometer. For each section, 15 different lengths were measured. Fifteen thickness measurements were made to calculate the average thickness for each rat.

Statistical analysis

Statistical analysis was carried out using SPSS 16 (SPSS/PC-16, SPSS Inc, USA). Data distribution was checked using the Kolmogorov-Smirnov test. The data that were normally distributed were analyzed using parametric techniques. Two-group comparisons were made using the t-test, whereas multiple-group comparisons were made using one-way analysis of variance (ANOVA). To compare sex ratio, the K^2test was performed. The results were expressed as mean ± S.E. (standard error), and *P*-values less than 0.05 were considered significant.

Results

Effect of pre-gestational stress on sex ratio in offspring

A significant difference was detected among the groups regarding the sex ratio of rat pups (Fig. 1). There was a significant difference in the number of male and female pups between groups ($p = 0.034$, K^2).

Effects of stress during spermatogenesis on male reproductive function

Effects of stress during spermatogenesis on sperm parameters are summarized in Table 1. Significant differences were seen in the percentages of progressive, non-progressive, and no-motility categories of sperm movement. Also, significant differences were detected in the number and morphology of sperm among experimental groups. A representative of sperm morphology is illustrated in Fig. 2.

Histological observations in testis parameters

SI and STET were significantly decreased in stressed groups compared with the control group ($p = 0.016$ and $p < 0.001$,

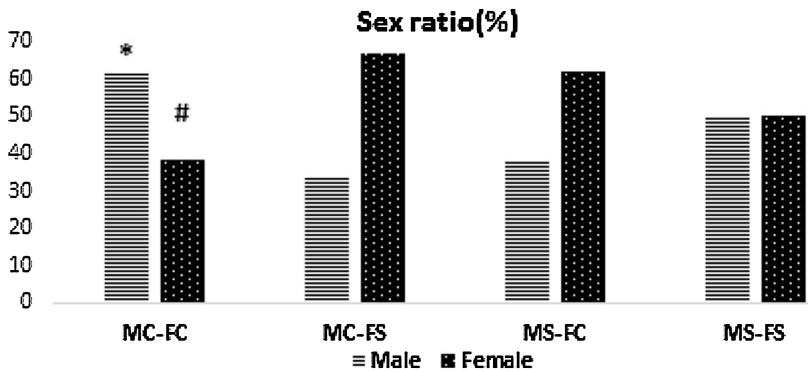

Fig. 1. Effect of pre-gestational exposure to predatory stress on sex ratio. There was a significant difference between male and female pups in Mc-Fc, Mc-Fs, and Ms-Fc groups ($p = 0.034$, K^2); * indicates a significant difference with male pups in all other groups; # indicates a significant difference with female pups in all other groups.

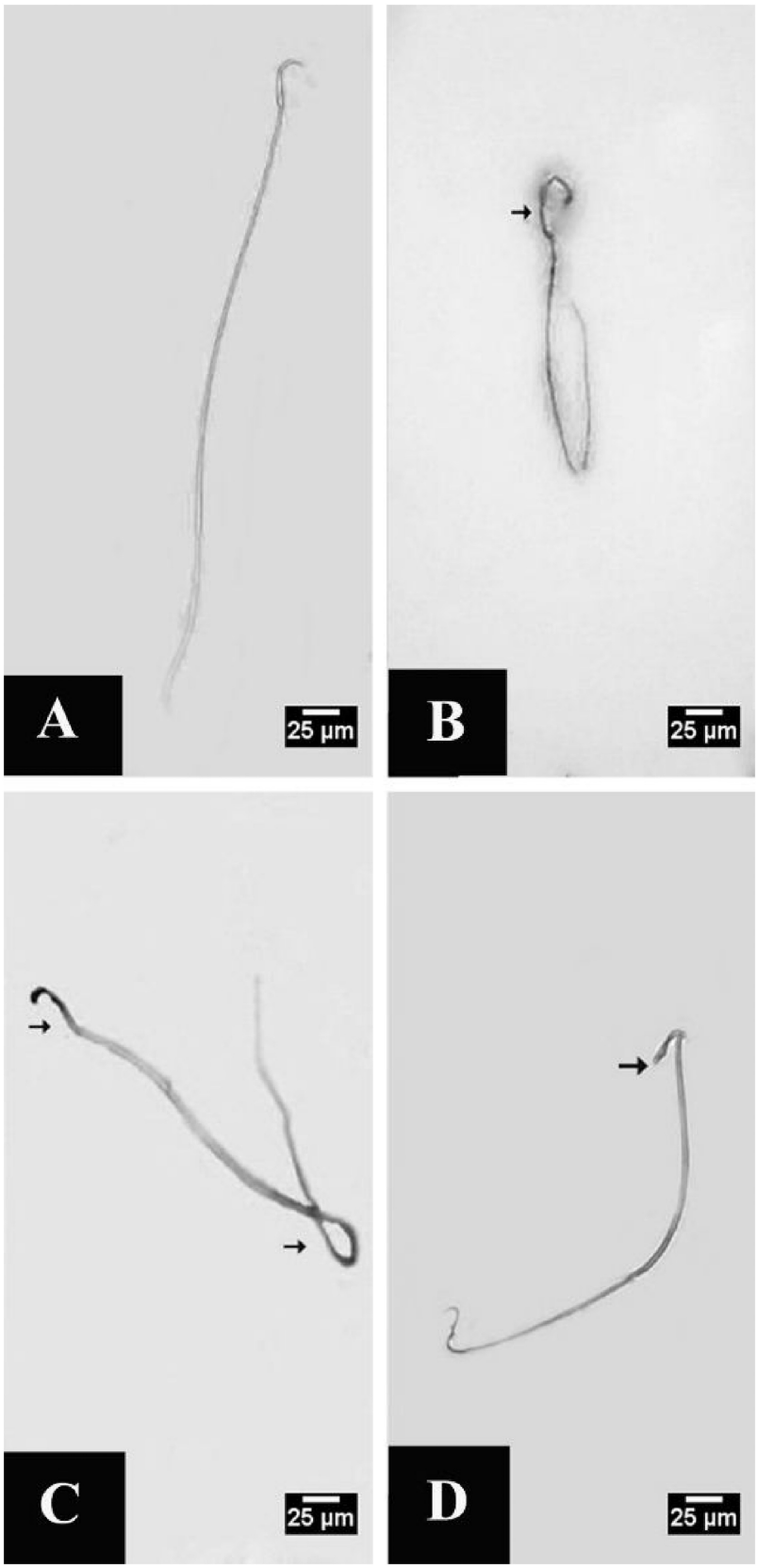

Fig. 2. Eosin nigrosin staining of sperms in adult Wistar rat sujected to predatory stress for 50 consecutive days. (A) a normal sperm; (B, C, and D) different forms of sperm morphological abnormalities in stressed rat.

Table 2
Different parameters of testis in control and stressed rats.

Sperm parameters	Control	Stressed	*P*
SI (%)	99.28 ± 0.71	85.62 ± 4.57	= 0.016
STET (μm)	108.98 ± 3.74	78.08 ± 2.69	<0.001
Sloughing (%)	10.71 ± 0.71	31.25 ± 3.23	<0.001
DST (μm)	302.93 ± 10.89	295.44 ± 6.44	>0.05
Detachment (%)	12.14 ± 1.48	41.25 ± 1.48	= 0.002

SI: spermatogenesis index, STET: seminiferous tubule epithelium thickness, DST: diameter of the seminiferous tubule.

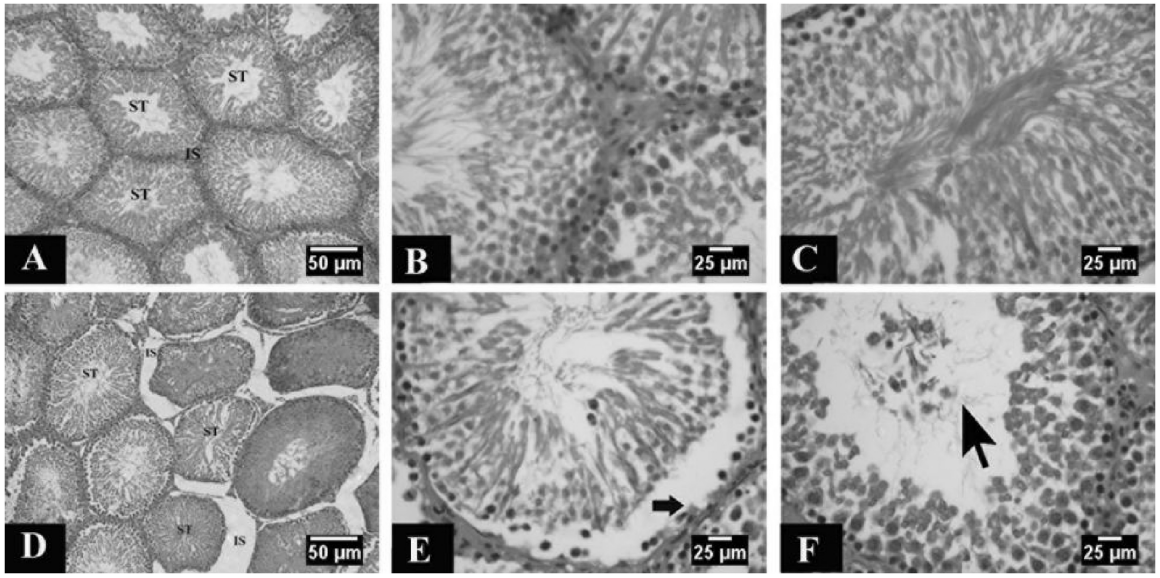

Fig. 3. Cross-sections from the testicular tissue of rats; (A, B, and C) control rat: Note normal tissue and seminiferous tubules (ST) with sperm in their lumen and normal interstitial space (IS). (D, E, and F) stressed rat: The ST have irregular shapes. Note the edema in IS. Arrow in Panel E shows the detachment of germinal epithelium and, in Panel F, shows an ST lacking sperm in lumen and sloughing of germ cells into the lumen. H&E staining.

Table 3
Effects of stress during oogenesis on vaginal epithelium in control and stressed rats.

Vaginal epithelial thickness (μm)	Control Mean ± SE	Stressed	*P*
Estrus Phase	137.98 ± 3.52	88.49 ± 5.36	0.002
Metestrus Phase	97.38 ± 9.51	49.67 ± 4.05	0.01
Diestrus Phase	52.79 ± 4.94	27.21 ± 2.53	0.01

respectively). Sloughing and detachment were significantly increased in stressed groups compared with control rats ($p < 0.001$ and $p = 0.002$, respectively), while the diameter of ST was decreased in the stressed rats but not significantly (Table 2 and Fig. 3).

Effects of stress during oogenesis on female reproductive function

A significant decrease was observed in the vaginal epithelium of estrus ($p = 0.002$), metestrus ($p = 0.01$), and diestrus ($p = 0.01$) phases in stressed rats compared with the controls (Table 3 and Fig. 4). However, there was no significant difference in vaginal pH between control and stressed rats.

Effects of pre-gestational stress on sex hormone levels

In adult males, a significant decrease was observed in the serum testosterone levels of stressed rats compared with the controls ($p = 0.018$). In adult females, a significant decrease was observed in the serum 17-beta estradiol levels of stressed rats compared with the controls ($p = 0.021$) (Fig. 5). Serum 17-beta estradiol in female pups and testosterone levels in male pups were decreased in the stress groups, but not significantly.

Discussion

In the present study, the impact of pre-gestational (during spermatogenesis and oogenesis) predatory stress on sex ratio and male and female reproductive systems was investigated. This study is unique because both parents were stressed simultaneously before conception. The main finding of the study was that exposure to stress led to a significant decrease in the number of male offspring in the stressed groups. Parameters of sperm (concentration, motility, and morphology), parameters of testicles (STET, sloughing, and detachment), vaginal epithelial thickness, estradiol in adult females, and testosterone in adult males were decreased in stressed rats compared with the controls.

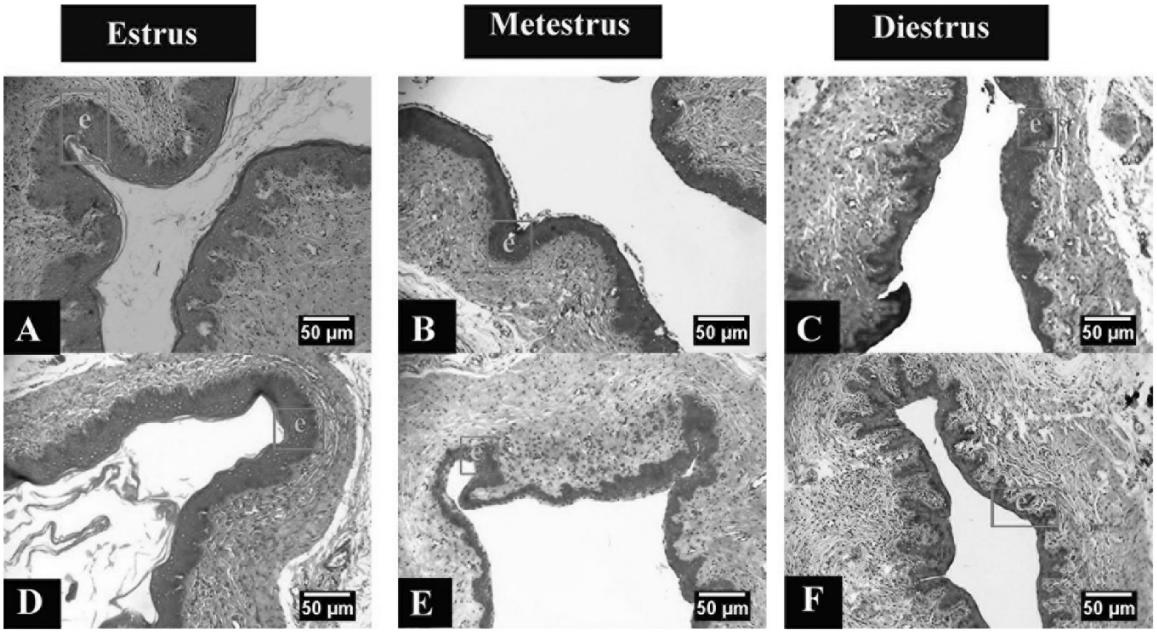

Fig. 4. Histological analysis of vagina, H&E staining. A, B, and C are from the control rat, indicating estrus, metestrus, and diestrus phases, respectively; D, E, and F are from the stressed rat, showing estrous, metestrus, and diestrus phases, respectively. A remarkable decrease was observed in the thickness of vaginal epithelium in all phases of stressed rats compared with the controls; e = epithelium.

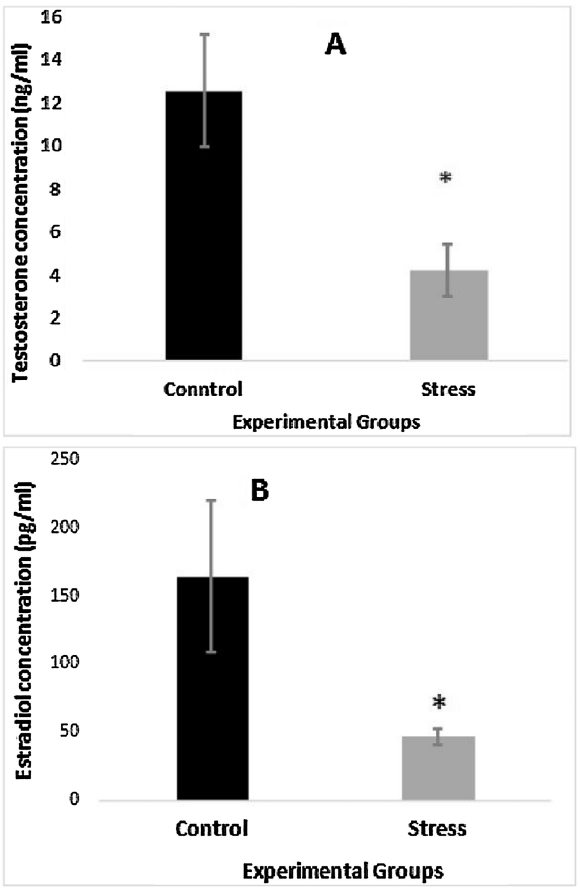

Fig. 5. Effect of pre-gestational exposure to predatory stress on sex hormone levels in male and female adult rats; (A) * indicates that serum testosterone levels decreased significantly in stressed rats compared to controls ($p = 0.018$). (B) * indicates that serum estradiol levels decreased significantly in stressed rats compared to controls ($p = 0.021$).

Effects of stress during spermatogenesis on male reproductive function

Experimental evidence indicates that environmental factors including chemical, physical, or emotional ones may adversely affect testicular functions (Rai et al., 2003). Spermatogenesis is a complex cellular process responsive to developmental factors and highly sensitive to chemical toxicants and environmental stress. Androgens (particularly testosterone) are vital for the initiation of spermatogenesis and sperm maturation (Maines and Ewing, 1996). Chronic exposure to stress is recognized to cause a diversity of pathophysiological changes in the neuroendocrine system, ensuing altered steroidogenesis and spermatogenesis (Shukla et al., 2010). Anterior pituitary gonadotropins and testicular androgens are crucial for the growth and differentiation of somatic cells in the testes and for the initiation and maintenance of spermatogenesis (Tapanainen et al., 1993). Cortisol in humans and corticosterone in rodents have been designated as stress hormones. Gonadal and sexual dysfunctions are linked with raised circulating cortisol levels. Glucocorticoids levels in circulation increase rapidly in response to stress, causing testicular involution followed by a significant drop in testosterone secretion (Mahdi et al., 2011). The diminished androgenic status of stressed rats may be responsible, at least in part, for depressing spermatogenesis, since the stimulatory action of both gonadotropins and testosterone is required to initiate and preserve the process. In addition to hormonal control, local regulatory interactions occur between all testicular cell types and these cell-cell signaling, involving growth factors, intermediate the cell growth and differentiation essential for the initiation and maintenance of spermatogenesis. Stress may also interfere with elements of this precise paracrine control, impairing the spermatogenic process (Almeida et al., 1998; Ralph et al., 2016). Elevated psychological stress can be linked with augmented oxidant production, and long-term exposure to stress factors may increase the generation of reactive oxygen species (ROS) (Mahdi et al., 2011). When the production of ROS is high, it can lead to oxidative damage to spermatozoa (Kalantari Hesari et al., 2015). Augmented ROS levels may lead to an imbalance in oxidant/anti-oxidant ratio, leading to increased lipid peroxidation

and resulting in sperm membrane damage and its subsequent dysfunction (Shukla et al., 2010). Our result is consistent with the existing literature indicating that the quality of sperm and testicular parameters significantly decrease after 50 days of predatory stress (Rai et al., 2003; Shukla et al., 2010).

Effects of stress during oogenesis on female reproductive function

The hypothalamus-pituitary-adrenal (HPA) axis exerts an inhibitory effect on the female reproductive system. The corticotropin-releasing hormone inhibits the secretion of the hypothalamic gonadotropin-releasing hormone, while glucocorticoids suppress pituitary luteinizing hormone (LH), ovarian estrogen, and progesterone secretion and render target tissues resistant to estradiol (Kalantaridou et al., 2004). Plasma estradiol levels in female rats decrease in response to chronic stress (Galea et al., 1997). All over a woman's life cycle, the vaginal epithelium experiences changes in response to the level of circulating estrogen (Bachmann and Nevadunsky, 2000). Estradiol-17b (E2) stimulates uterine and vaginal epithelial proliferation *in vivo*. E2 also plays an essential role in other features of uterine and vaginal growth and adult sexual function and is required for normal epithelial morphogenesis, cell differentiation, and secretory activity in these organs (Cooke et al., 1998). The results of our study, similar to findings of previous studies, showed a significant reduction in the E2 of stressed rats and, consequently, a reduction in the thickness of vaginal epithelium.

Effects of pre-gestational stress on sex ratio

Studies focusing on epigenetics and endocrine disturbance may help explain how environmental factors interfere with human reproduction (Terrell et al., 2011). It has been reported that parents under deprivation and stress are more likely to have female birth (Hansen et al., 1999; Song, 2015; Pérez-Crespo et al., 2008). Exposure to disasters including flood and earthquake has been associated with a higher sex ratio in female offspring (proportion of females) conceived at the time of the exposure episode (Hansen et al., 1999). There are several mechanisms by which environmental factors might decrease the male-to-female ratio in men (Terrell et al., 2011). Numerous studies stated that physical and psychological stressors activate the HPA axis and lead to a noticeable and persistent rise in plasma glucocorticoid levels (Ebrahimi et al., 2014; Gholipoor et al., 2017; Hashemi et al., 2016; Saboory et al., 2015; Tavassoli et al., 2013). In men, stress leads to adrenocorticotrophic hormone (ACTH) secretion, reducing gonadotropins and therefore lowering testosterone levels, whereas in women, ACTH leads to the activation of adrenals and increased testosterone levels. Thus, this difference between the endocrine responses of men and women to stress may underlie the stated difference between sex ratios of offspring of stressed men and women (James, 1996). It has been noted that ACTH-induced increases in serum levels of glucocorticoids suppresses the episodic secretion pattern of LH and testosterone and decreases the basal secretion rate of testosterone (McGrady, 1984). It is suggested that high paternal levels of testosterone are linked with the production of male offspring (James, 2008). Meanwhile, it has been reported that the glutathione peroxidase-like protein specific to mouse epididymis (GPP) is regulated by androgens. This protein prevents oxidative damage to sperms. While the regulation takes place *via* a classic steroid hormone receptor system, any possible difference in X- and Y-sperm reaction leads to differences in comparative fertility in relation to androgens (particularly testosterone) levels. Therefore, paternal hormone levels can be associated to sex ratio in rats and humans (Krackow, 1995). Previous studies have showed that stress has a negative influence on numerous parameters related with semen quality, including sperm count, motility, and morphology (Shukla et al., 2010). In a study conducted on red deers, an association was found between sperm quality and the sex ratio of the offspring (Pérez-Crespo et al., 2008). In humans, similarly, it was shown that factors which decrease male fertility are linked with a decrease in the proportion of male births. Moreover, paternal exposures to environmental threats decrease the percentage of Y-bearing sperms in the semen or lower the capability of Y-bearing sperms to reach or fertilize the oocyte (Terrell et al., 2011). It seems that this altered environment might differently affect the function of sperms carrying X or Y chromosomes (Pérez-Crespo et al., 2008). The vulnerability of the Y chromosome to DNA omissions is likely related to its inability to contribute to recombination repair (Aitken and Krausz, 2001).

In addition, studies specify that stress can alter the patterns of cortisol excretion during the estrous cycle which eventually affects the hormonal profile in critical phases of the reproductive process (Liu et al., 2012). In fact, female rats show decreased levels of estradiol in response to a chronic stressor (Shors et al., 1999), and high levels of estrogens are associated with the production of male offspring (James, 2008). Maternal predatory stress during the oocyte pre-maturation phase decreases oocyte developmental potential at both pre- and post-implantation stages (Liu et al., 2012). Therefore, this might lead to a disproportionate loss of XY embryos (Terrell et al., 2011). On the other hand, male fetuses grow larger and thus need a larger investment of resources by the mother, and may not adapt their development to a stressful intrauterine environment. Instead, female fetuses use fewer resources as they develop and are supposed to have reduced growth and demands on the mother in response to maternal stress (Pickering et al., 1987). Our study is unique in this context because we used both parents stressed status along with single parent (mother or father) stressed. According to existing literature, both paternal and maternal stress alters the offspring sex ratio in favor of females (Liu et al., 2012; Terrell et al., 2011). Our study revealed that this effect is more potent when just mother was stressed (group Mc-Fs).

Conclusions

It can be concluded that pre-pregnancy stress alters the sex ratio of the offspring. The ratio of male to female decreases in stressed subjects, confirming the existing literature and more precisely revealing the crucial importance of the mental health of both parents in the preconception period. The stress during spermatogenesis and oogenesis has harmful effect on the genital system; in males, stress reduces the quality of sperm, testicular parameters, and concentration of testosterone in blood. Also, the stress during oogenesis reduces the thickness of vaginal epithelium in all phases of the estrous cycle and lowers the concentration of 17-beta-estradiol in blood.

Conflict of interests

The authors have no conflict of interests to declare regarding the study described in this article and the preparation of the article.

Acknowledgements

This study was supported by the Neurophysiology Research Center, Urmia University of Medical Sciences, Urmia, Iran. This article was extracted from Maryam Mahmoodkhani's thesis for completing the requirements of a Master's Degree in physiology.

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