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Pregnancy Rate in Female Mice Exposed to Forced Swimming Stress

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ABSTRACT

The present experimental study was designed to investigate to elucidative effect of forced swimming stress on female mice fertility with emphasis on oocyte number and their quality and ultimately their *in vitro* fertilization capacity. A total 30 adult NMRI (Noda Medical Research Institute) mice randomly divided into two equal groups (n = 15); 1) control and 2) experimental groups. The control group remained in their cages, but experimental group was submitted to forced swimming for 3 min in water at 32°C daily for 50 days. The NMRI female mice in two group of study who mention above were administered intra-peritoneally with 5 IU pregnant mare serum gonadotropin (PMG) for superovulation. This was followed 46-48 h later by the intraperitoneal administration of 5 IU Human Chorionic Gonadotropin (HCG). Mice were euthanized 12-14 h after HCG injection by cervical dislocation method and oocytes collected from fallopian tube. Then count and quality of oocyte were assessed. In order to evaluated of fertilization capacity, a pre-incubated capacitated sperm was gently added to the freshly collected ova of two groups of study and two-cell embryos was counted 24-26 h after completion of fertilization *in vitro*. The fertilization capacity of oocyte of exposed to forced swimming stress was significantly lower than control groups ($p<0.05$). The plasma level of FSH and LH was significantly increased in stress mice group ($p<0.05$). The data of this present study clearly showed that forced swimming stress do not alter number of oocyte extraction but quality of extracted oocyte significantly decrease in mice exposed to forced swimming stress.

Key words: Stress, oocyte quality, LH, FSH

INTRODUCTION

A couple is considered infertile if the woman does not conceive a child after one year of unprotected, well-timed intercourse, or she has been unable to carry a pregnancy to a live birth. In the United States about 10% of couples are affected by infertility (Philippov *et al*., 1998). According to the American Society for Reproductive Medicine, roughly one-third of infertility cases can be attributed to male factors and another one-third to factors that affect women. For the remaining infertile couples, infertility is caused by a combination of problems in both partners (about 13%) or is unexplained (about 10%) (Philippov *et al*., 1998; Sheiner *et al*., 2002). The most common cause of female infertility is an ovulation disorder. Other causes of female infertility include blocked fallopian tubes, polycystic ovary syndrome (PCOS) and endometriosis (Maubon *et al*., 2008). Psychological stress has been compromised as one of the major causes of

idiopathic infertility in both men and women (Shahid, 2009; Wischmann *et al*., 2009; Akizuki and Kai, 2008; Damti *et al*., 2008; Moreira *et al*., 2005). Many studies have investigated psychological causes affect male factor infertility (Zorn *et al*., 2008; Clarke *et al*., 1999; Hjollund *et al*., 2004a, b; Sheiner *et al*., 2003; Pook *et al*., 2005), but it remains difficult to tease out stress as a cause or consequence of infertility. A diversity of stress factors such as microorganisms, hyperthermia and exposure to heavy metals inhibit male reproductive functions and spermatogenesis (Ozawa *et al*., 2002). After implementation of the stressful stimuli such as prolonged immobilization and forced swimming stress, similar effects were observed by Almeida *et al*. (2000) and Mingoti *et al*. (2003). In erlear study, it is demonstrated that forced swimming is one of the main stressful factors in the time course equal spermatogenesis period i.e., 48-50 days (Saki *et al*., 2009). In the rat will be significantly effective to reduce the number and motility of sperm as well as the *in vitro* fertilization capacity (Saki *et al*., 2009). Previous study showed that the rate of pre-and post-implantation loss in female rats mated with stressed male rats significantly increased (Saki *et al*., 2010). With our literature review, we reached the conclusion that no published report on the effect of forced swimming stress on the of female mice fertility. So it was decided to be a study done in females mice with emphasis on oocyte number and their quality and ultimately their *in vitro* fertilization capacity.

MATERIALS AND METHODS

Animals: This study was conducted from March 2009 to November 2009. A total 30 adult NMRI (Noda Medical Research Institute) female mice 8-10 weeks of age, weighing 55±6.6 g were purchased from Laboratory Animals Care and Breeding Center of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. All mice were randomly divided into two equal groups (n = 15); (1) control and (2) experimental groups. All animals were housed individually per cage under a 12 h light/dark cycle, 20±2°C temperature and 60-65% humidity-controlled room with food and water *ad libitum*. All procedures were approved by international guidelines and by the Institute Research Ethics and Animal Care and Use Committee of Ahvaz Jundishapur University of Medical Sciences.

Experimental design: As the control group remained in their cages, the experimental group was submitted to forced swimming for 3 min in water at 32°C daily for 50 days. Stress was sized up by the hot-plate test after the last stressing session (Mingoti *et al*., 2003). In the hot-plate, the plate temperature was 52°C and maximal cut-off time was 60 sec. The latency time for the hind licking after exposure to the hot-plate surface was measured and the increase in relation to control was considered to be an index of the antinociceptive effect.

Oocytes collection procedure: The NMRI female mice in two group of study who mention above were administered intra-peritoneally with 5 IU pregnant mare serum gonadotropin (PMG) for superovulation. This was followed 46-48 h later by the intraperitoneal administration of 5 IU Human Chorionic Gonadotropin (HCG). Mice were euthanized 12-14 h after HCG injection by cervical dislocation method. After disinfection with 70% alcohol and opening the abdomen wall, the Y-shaped uterus, ovaries and oviducts were identified. The oviducts were excised as follows: Clamping cornuas, dissecting the peritoneum and fat between ovary and tube and then cutting the fallopian tube from the proximal end and cumulus-oocyte complexes were collected in KSOM medium. The granulosa cells of oocytes were removed by pupating in KSOM medium containing 80 IU mL^{-1} hyaluronidase and mature oocytes obtained then the count and quality of oocyte were assessed.

Quality assessment of oocytes: Morphologic evaluation was performed using an inverted microscope at ×200 magnification. Oocyte morphology classification was established in accordance with the main observed morphological characteristics. They were grouped as follows:

- Healthy oocyte with a homogenous and clear cytoplasm, a normal polar body, perivitelline space and shape
- Absence of the first polar body in the perivitelline space
- Fragmented first polar body
- Abnormal perivitelline space
- Fragmented ooplasm
- Other problems: lysed ooplasm or not spherical oocyte
- Multiple abnormalities (at least two abnormalities) (Kundt *et al*., 2009)

Sperm extraction: Males of proven fertility were euthanized and the cauda epididymis removed and immediately inserts into a 150 μL drop of TYH medium+5 mg mL^{-1}. Bovine Serum Albumin (BSA) under mineral oil (Sig., embryo-tested, cat. No. M8410). The epididymis contents were squeezed out. The spermatozoa were extracted from the caudal part of epididymis and incubated for 60 min at 37°C in 5% CO_2 in humidified air for the purpose of capacitation (O'Flaherty *et al*., 2005).

IVF method: *In vitro* fertilization was carried out in drops of KSOM medium plus, 5 mg mL^{-1} Bovine Serum Albumin (BSA) under mineral oil. A pre-incubated capacitated sperm was gently added to the freshly collected ova of two groups of study. The combined sperm-oocyte suspension was incubated for 4-6 h under a condition of 5% CO_2 and 37°C temperature. The ova were then washed through several changes of medium and finally incubated in drops of KSOM+5 mg mL^{-1} BSA under mineral oil. Fertilization was assessed by recording the number of two-cell embryos 24-26 h after) completion of fertilization *in vitro* (Saki *et al*., 2006).

Hormone assay method: Blood plasma FSH and LH concentration were measured in each sample by radioimmunoassay (RIA) using two commercial Kits (Onclin *et al*., 2002).

Statistical analysis: Data are stated as a Means±SD and percentage. The statistical significance of difference between the control and experimental groups was determined by the chi-square. Differences between the means were considered to be significant when $p<0.05$ was achieved.

RESULTS AND DISCUSSION

In this study 15 adult female mice with 8 to 10 weeks old were used in each group of study. As shown in Table 1 the total extracted oocyte was 163 and 179 for control and stressed mice, respectively. The difference between two groups of mouse study was not significant ($p>0.05$).

The 96.64 and 57.54% of total oocyte extraction were healthy in control and stress mice groups, respectively. Statistical analysis showed the significant differences between two groups of study ($p<0.05$). There was difference in percentage of non healthy oocyte extraction of two groups of study (34.46% in control group versus 42.46% in stress mice group) ($p>0.05$). The fertilization capacity of oocyte of exposed to forced swimming stress was significantly lower than control groups ($p<0.05$). The plasma level of FSH and LH was significantly increased in stress mice group ($p<0.05$).

Table 1: Effect of forced swimming stress on number, quality, fertilization capacity of oocyte female mouse and sex hormones

	Animal study group (n = 15)		
Variables	Control	Experimental	p-value
Total extracted oocyte	163 Per uterus:10.86	179 Per uterus:11.93	0.070
Healthy oocyte (%)	107 (96.64%)	103(57.54%)	0.003
Non-healthy	56 (34.46%)	76(42.46%)	0.030
In vitro fertilization rate (%)	50 (30.67%)	140(78.21%)	0.008
FSH level (mIU mL^{-1} plasma)	0.21±0.03	0.11±0.04	0.010
LH level (mIU mL^{-1} plasma)	0.14±0.02	0.09±0.03	0.040

In the industrialized world, approximately 12% of couples suffer from infertility. Approximately 40% of the causes of infertility are attributed to women (Damti *et al*., 2008). A great deal of research in reproduction has revealed that the decrease in male and female fertility over the last few decades may be due to harmful environmental influences, pollutants and stress (Kaneko *et al*., 2004). The present experimental study was designed to investigate to elucidative effect of forced swimming stress on female mice fertility with emphasis on oocyte number and their quality and ultimately their *in vitro* fertilization capacity. The data of this present study clearly showed that forced swimming stress do not alter number of oocyte extraction but quality of extracted oocyte significantly decrease in mice exposed to forced swimming stress. As our best knowledge this report will be given for the first time. In this study, we observed that the rate of fertilization in oocyte extracted from mice exposed to forced swimming stress was decreased. This result may due to increase of the non healthy oocyte. Previous study showed that the quality of oocytes has the greatest influence on results of the monospermic fertilization, early development and implantation of embryos. Therefore, the quality of oocytes can be a determining factor in the fertilization of oocyte, culture of high quality embryos and treatment of infertility (Marteil *et al*., 2009). These findings are consistent with the results of present study. Under stimulation by the excess of pituitary Luteinizing Hormone (LH) *in vivo*, the oocyte reinitiates meiosis, nucleus of the oocyte changes its structure and then nuclear membrane of oocyte disappear (Fan and Sun, 2004). The microtubules become organized into a bipolar spindle and all chromosomes align at the cell equator. The first meiotic division continues in oocyte; after this division, the first polar body separates and it enters the perivitelline space. Then the second meiotic division takes place and stops in metaphase II. This process is known as the maturation of oocyte nucleus (Sun and Nagai, 2003). In order that the oocyte would be successfully fertilized and a new body would develop, the nucleus and cytoplasm of oocyte must be mature at the same time (Krisher, 2004). The results of this study showed that plasma level of LH and FSH significantly decrease in mice exposed to forced swimming. This finding supported the study of Damti *et al*. (2008). In conclusion may we can conclude that LH and as well as FSH have an important effect on oocyte quality.

CONCLUSION

The present data demonstrate that forced swimming, when applied to adult female rats, quality of extracted oocyte significantly decrease. This result may raise the attention to effect of stress in humans in terms of sports training and stress from day to day life.

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REFERENCES

Akizuki, Y. and I. Kai, 2008. Infertile Japanese women's perception of positive and negative social interactions within their social networks. Hum. Reprod., 23: 2737-2743.

Almeida, A.S., W.G. Kempinas and T.L.L. Carvalho, 2000. Sexual behavior and fertility of male rats submitted to prolonged immobilization-induced stress. Braz. J. Med. Biol. Res., 33: 1105-1109.

Clarke, R.N., S.C. Klock, A. Geoghegan and D.E. Travassos, 1999. Relationship between psychological stress and semen quality among *in vitro* fertilization patients. Hum. Reprod., 14: 753-758.

Damti, O.B., O. Sarid, E. Sheiner, T. Zilberstein and J. Cwikel, 2008. Stress and distress in infertility among women. Harefuah, 147: 256-60-276.

Fan, H.Y. and Q.Y. Sun, 2004. Involvement of mitogen-activated protein kinase cascade during oocyte maturation and fertilization in mammals. Biol. Reprod., 70: 535-547.

Hjollund, N.H., J.P. Bonde, T.B. Henriksen, A. Giwercman and J. Olsen, 2004a. Reproductive effects of male psychologic stress. Epidemiology, 15: 21-27.

Hjollund, N.H., J.P. Bonde, T.B. Henriksen, A. Giwercman and J. Olsen, 2004b. Job strain and male fertility. Epidemiology, 15: 114-117.

Kaneko, M., M. Ali and H. Ushijima, 2004. Relationship between decreasing fertility during the post-war period and maternal age in a Japanese population. Tohoku J. Exp. Med., 202: 221-226.

Krisher, R.L., 2004. The effect of oocyte quality on development. J. Anim. Sci., 82: E14-E23.

Kundt, M.S., C. Martinez-Taibo, M.C. Muhlmann and J.C. Furnari, 2009. Uranium in drinking water: Effects on mouse oocyte quality. Health Phys., 96: 568-574.

Marteil, G., L. Richard-Parpaillon and J.Z. Kubiak, 2009. Role of oocyte quality in meiotic maturation and embryonic development. Reprod. Biol., 9: 203-224.

Maubon, A., M. Pouquet, P. Piver, N. Mazet, M. Viala-Trentini and J.P. Rouanet, 2008. Imaging of female infertility. J. Radiol., 89: 172-183.

Mingoti, G.Z., R.N. Pereita and C.M.R. Monteiro, 2003. Fertility of male adult rats submitted to forced swimming stress. Brazil. J. Med. Biol. Res., 36: 677-681.

Moreira, S.N., F.F. Tertulino, S.C.A. Nogueira, A.G. Oliveira, G. Tomaz and G.D. de Azevedo, 2005. Psicological aspects in infertile women. Acta Cirurgica Brasileira, 1: 152-158.

Onclin, K., B. Murphy and J.P. Verstegen, 2002. Comparisons of estradiol, LH and FSH patterns in pregnant and nonpregnant beagle bitches. Theriogenology, 57: 1957-1972.

Ozawa, N., N. Goda, N. Makino, T. Yamaguchi, Y. Yoshimura and M. Suematsu, 2002. Leydig cell-derived heme oxygenase-1 regulates apoptosis of premeiotic germ cells in response to stress. J. Clin. Invest., 109: 457-467.

O'Flaherty, C., E. Breininger, N. Beorlegui and M.T. Beconi, 2005. Acrosome reaction in bovine spermatozoa: Role of reactive oxygen species and lactate dehydrogenase C4. Biochim. Biophys. Acta, 1726: 96-101.

Philippov, O.S., A.A. Radionchenko, V.P. Bolotova, N.I. Voronovskaya and T.V. Potemkina, 1998. Estimation of the prevalence and causes of infertility in Western Sibria. Bull. Word Health Organ., 76: 183-187.

Pook, M., B. Tuschen-Caffier, J. Kubek, W.B. Schill and W. Krause, 2005. Personality, coping and sperm count. Andrologia, 37: 29-35.

Saki, G., Z. Safikhani, A. Sobhani and M. Salehnia, 2006. The *in vitro* fertilization rate of mouse ova in the absence or presence of recombinant human leukemia inhibitory factor. Pak. Med. Sci., 22: 438-441.

Saki, G., F. Rahim and K. Alizadeh, 2009. Effect of forced swimming stress on count, motility and fertilization capacity of the sperm in adult rats. J. Hum. Reprod. Sci., 2: 72-75.

Saki, G., F. Rahim and A.O. Waysi, 2010. Effect of forced swimming stress on *in vivo* fertilization capacity of rat and subsequent offspring quality. J. Hum. Reprod. Sci., (In Press).

Shahid, S., 2009. Depression in infertile couples. J. Coll. Physicians Surg. Pak., 19: 395-396.

Sheiner, E.K., E. Sheiner, R. Carel, G. Potashnik and I. Shoham-Vardi, 2002. Potential association between male infertility and occupational psychological stress. J. Occup. Environ. Med., 44: 1093-1099.

Sheiner, E., E.K. Sheiner, G. Potashnik, R. Carel and I. Shoham-Vardi, 2003. The relationship between occupational psychological stress and female fertility. Occup. Med., 53: 265-269.

Sun, Q.Y. and T. Nagai, 2003. Molecular mechanisms underlying pig oocyte maturation and fertilization. J. Reprod. Dev., 49: 347-359.

Wischmann, T., H. Scherg, T. Strowitzki and R. Verres, 2009. Psychosocial characteristics of women and men attending infertility counseling. Hum. Reprod., 24: 378-385.

Zorn, B., J. Auger, V. Velikonja, M. Kolbezen and H. Meden-Vrtovec, 2008. Psychological factors in male partners of infertile couples: Relationship with semen quality and early miscarriage. Int. J. Androl., 31: 557-564.