Abstract—Studies show that restraint stress influences male reproductive system. The main purpose of this study was to study the effects of restraint stress on serum LH, FSH or testosterone level and testes tissue in rats.

Methods: Male Wistar rats were randomly divided into control, acutely or chronically immobilized animals of 5 in each group. In chronically or acutely immobilized groups, animals were immobilized for 2h/day or 8h/day for a period of 3 weeks or one week, respectively. Blood samples were collected using cardiac puncture method. Following serum collection, LH, FSH and testosterone level was measured by radioimmunoassay method. Testes tissue was also examined histologically using HE staining method. Data were statistically analyzed and compared between groups using ANOVA.

Results: The results indicated that serum LH and testosterone level was significantly decreased in acutely or chronically immobilized rats compared with control animals (P<0.01), however serum FSH was not changed in immobilized rats compared with control rats. Deformed seminiferous tubules, reduced cellular concentration, decreased number of spermatocytes, spermatids and spermatozons was observed in restrained rats compared to control animals.

Conclusion: Our findings show that restraint stress has inhibitory effects on male reproductive system may lead to male reproductive failure.

Keywords-FSH, LH, Restraint Stress, Rats, Testosterone.

I. INTRODUCTION

IMMOBILIZING and restraining can influence many physiological aspects of organism and are considered as stress condition which is followed by alterations in body systems including reproductive system. Such stress has capability to alter normal function of hypothalamus-pituitary-endocrine glands axes leading to changes in releasing of hormones and their serum levels [1],[2].

It has also been shown that immobilization stress influences nitric oxide synthase activity resulting in altered plasma levels of nitrite oxide (NO) which in turn can play a part in changing the reproductive system normal function and also corticosteroids secretion [3],[4].

Studies show that chronic mild immobilization can act on secretion of releasing hormones from anterior pituitary by which influence thyroid gland function [5], [6], which in turn can influence testes metabolism and so function. Although studies indicate that restraint stress can lead to reduced serum levels of androgens [7] and reduced spermatogenesis [8], there are reports indicating that immobilization has no effect on testes function [2].

According to conflicting data relating to effects of restraint stress on male reproductive system, this study was performed to clarify the effects of chronic or acute immobilization on serum LH, FSH or testosterone level and testes tissue in rats.

II. MATERIAL AND METHODS

A. Animals

Adult Wistar rats weighting 200±30g were purchased and raised in our colony from an original stock of Pasteur institute (Tehran, Iran).The temperature was at 23±2°C and animals kept under a schedule of 12h light:12h darkness (light on at: 08:00 a.m.) with free access to water and standard laboratory chow. Care was taken to examine the animals for general pathological symptoms. Food was withheld for 12-14h before death. This study was performed according to ethical guidelines relating to working with laboratory animals [9].

B. Materials

The commercially available solid phase RIA kit (Immunotech A Beckman Coulter/ Ref.2121) was used for LH, FSH and testosterone assay.

C. Protocol of study

Male Wistar rats were randomly divided into control, acutely or chronically immobilized animals of 5 in each group. Based on previous studies [10], standard restrainer was used to immobilize animals. In chronically or acutely immobilized groups, animals were immobilized for 2h/day or 8h/day for a period of 3 weeks or one week, respectively.

D. Processing of blood samples and testis tissue

Blood samples were collected in appropriate tubes by cardiac puncture technique 24h after the last treatment. After collection, the blood samples left to clot at room temperature for 15 minutes and then centrifuged at 2500 r.p.m for 15 minutes. The serum layer was then separated
and aliquoted into small test tubes and stored at -20°C until LH, FSH or testosterone determination. For testis histological studies, testes were removed and after fixation in Bouin’s solution, testis tissue was transferred into 70% ethanol before being processed for 17.5 h in an automated Shandon processor and embedded in paraffin wax. Sections of 5 µm thickness were cut, floated onto slides coated with 2% 3-aminopropyltriethoxy-silane and dried at 50°C overnight before being used for cell quantification studies.

E. Statistical analysis
All values are presented as mean ± S.E.M. Statistical significance was evaluated by one-way analysis of variance (ANOVA) test. Significance was measured using Fisher’s least significant for the exact P values and significant differences are noted in the results. The difference between groups was considered significant when α<0.05.

III. RESULTS

Table I presents LH, FSH and testosterone concentration in male rats. Serum LH and testosterone level was significantly decreased in rats enduring acute or chronic immobilization stress compared with control animals (P<0.001). FSH level was not significantly changed in acutely or chronically immobilized animals compared to control group.

TABLE I
SERUM LH, FSH AND TESTOSTERONE LEVEL IN MALE RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Testosterone (µM)</th>
<th>LH (u/ml)</th>
<th>FSH (u/ml)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.46 ±1.37</td>
<td>0.25 ± 0.18</td>
<td>8.60 ± 1.14</td>
<td>N.S</td>
</tr>
<tr>
<td>Acutely Immobilized</td>
<td>2.03±0.31</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>9.18 ± 3.00</td>
</tr>
<tr>
<td>Chronically Immobilized</td>
<td>2.03±0.41</td>
<td>&lt;0.001</td>
<td>0.16 ± 0.06</td>
<td>7.90 ± 1.51</td>
</tr>
</tbody>
</table>

Data are appeared as mean ± SEM. P values are versus control animals. NS indicates nonsignificant difference compared with control rats.

Our results show that seminiferous wall diameter was not significantly changed in acutely or chronically immobilized animals compared to control rats. There was also lower number of spermatozons in seminiferous tubes in acutely or chronically immobilized animals compared to control rats (P<0.001). Seminiferous tubes were also morphologically deformed in immobilized rats compared to control group (figure 1).

Fig 1. Seminiferous tubules in immobilized and control animals

III. DISCUSSION

In our study, acute or chronic immobilization caused to decreased serum LH and testosterone level, deformed seminiferous tubules and reduced cellular concentration and spermatogenesis in tubules. These findings support the concept that stress has inhibitory effects on male reproductive system. It has long been shown that many aspects of body neuroendocrine and physiological function are influenced by many types of stress [11],[12]. Stress can also suppress immune responses [10]. On the other hand, studies show that different stress modalities result in distinct steroid hormone responses by male rats [13]. In accordance with our study there are other reports indicating that immobilization stress reduces serum levels of testosterone or LH [7], [14], [15]. Other studies also have found that immobilization stress alters testes function and reduced spermatogenesis [8] as we indicated in our study. However, in contrast to our findings, there are reports showing that stress modality is a key factor in affecting on male reproductive system such that some types of immobilization stress does not influence reproductive function [2], [13].

Since stress inhibits thyroid gland function, reduced testes function following immobilization stress is likely appeared due to reduced activity of thyroid gland [5], [6] resulting in reduced metabolic rate in testes tissue. Restraint stress also influences hypothalamus secretions by which can alter hypophysis function resulting in reduced LH and testosterone level [14]. However, there are reports indicating that LH level is not reduced following restraint stress [3].
Inhibition of testes function following restraint stress may result from alteration in NO level or endogenous opioids such as endorphins or enkephalins. Studies show that nitric oxide synthase expression is sensitive to restraint stress [4]. The findings of investigators also indicate that opioid antagonists have a part in local regulation of testicular response to acute stress in adult rats [16]. Despite decreasing of serum LH level in our study, FSH level was not significantly changed in acutely or chronically immobilized rats compared to control animals. This may result from the short period of experiment in which the FSH level has not been changed significantly.

V. CONCLUSION

Our findings show that immobilization stress reduces male reproductive function which can provide male reproductive failure. Therefore, sedentary life which includes some aspects of restraint stress is biomedically important in male reproductive failure.

REFERENCES