Original Article

Bull Emerg Trauma 2015;3(4):122-127.

Bulletin of Emergency And Trauma

Effects of Unilateral Iatrogenic Vas Deferens Trauma on Fertility: An Experimental *In Vitro* Fertilization Mice Model Study

***Mohammad Babaei*[1], *Gholamreza Najafi*[1], *Ali Shalizar Jalali*[1*], *Mehdi Behfar*[2]**

[1]*Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*
[2]*Department of Surgery and Diagnostic Imaging, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

***Corresponding author:** *Ali Shalizar Jalali*
Address: Histology and Embryology Research Laboratories, Department of Basic Sciences, University of Urmia, Urmia, Iran. **Tel:** +98-44-32770508; **Fax:** +98-44-32771926
e-mail: ali_shalizar@yahoo.com; a.shalizar@urmia.ac.ir

Received: June 15, 2015
Revised: June 27, 2015
Accepted: July 7, 2015

ABSTRACT

Objective: To determine bilateral effects of unilateral iatrogenic vas deferens trauma (UIT) on epididymal sperm characteristics and *in vitro* fertilizing capacity in an experimental mouse model.
Methods: Experiments were performed on three equal groups each comprising six adult male mice. Following anaesthesia, UIT was induced by clamping left vas deferens with a mosquito clamp in fully locked fashion for 2 minutes in UIT group. Control-sham mice only had exposure of the left vas deferens through a groin incision. Control animals only received ceftriaxone (100 mg/kg) intraperitoneally at the day of experimental UIT induction. Ipsilateral and contralateral epididymal sperm characteristics and *in vitro* fertilizing capacity were evaluated after 35 days.
Results: UIT significantly decreased sperm concentration, motility and viability as well as fertilization, two-cell embryos, blastocysts and hatched blastocysts rates. Moreover, incidence of DNA damage and abnormality in spermatozoa was significantly higher in UIT group.
Conclusion: The findings suggest that a non-recognized iatrogenic vas deferens trauma may have detrimental effects on spermatozoa leading to infertility.

Keywords: *In vitro*; Fertilization; Mouse; Sperm; Trauma; Vas deferens.

Please cite this paper as:
Babaei M, Najafi GR, Shalizar Jalali A, Behfar M. Effects of Unilateral Iatrogenic Vas Deferens Trauma on Fertility: An Experimental *In Vitro* Fertilization Mice Model Study. *Bull Emerg Trauma*. 2015;3(4):122-127.

Introduction

Vas deferens iatrogenic injuries caused by involuntary medical interventions are serious complications of surgical procedures in the inguinal canal in children and adults [1,2].The average paediatric surgical practice is overflowing with operations in close proximity to the vas deferens. It is, therefore, very important to know what effects routine inguinal exploration have on the structures of the inguinal canal. Unintended transaction of the vas deferens is an evident catastrophe with lifelong sequels [3]. Inguinal hernia repair, one of the most frequent operations performed worldwide by general

and paediatric surgeons, is performed by using the open, laparoscopic or preperitoneal approach [4]. Transection or ligation related injuries account for about 25% of iatrogenic vassal injuries [5]. Most infant surgeries deal with inguinal pathology and during these surgical procedures the vas deferens and vessels are involuntarily manipulated. The accidental crushing of inguinal contents is at times unavoidable and the results unpredictable. It may not be as harmful as iatrogenic separation, but some sort of disturbance in the continuity of the vas may have a long-lasting functional effect. It has been proven that any slight disturbance in vas deferens muscular layer or mucosa will jeopardize fertility [6]. Epididymitis, trauma or iatrogenic injuries could be involved in vas deferens injuries. Surgical intervention-associated damages in vas deferens can happen during orchidopexy, hydrocele repair or inguinal herniotherapy [7]. It was found that remote inguinal hernia repair is a common finding in infertile men with unilateral vasal obstruction [8]. Moreover, previous studies have shown that unilateral vasal injuries have immune response-mediated detrimental effects on the contralateral testicular function [9,10].

In view of this, the main goal of this study was to elucidate bilateral effects of unilateral iatrogenic vas deferens trauma (UIT) on epididymal sperm characteristics and *in vitro* fertilizing capacity in an experimental mouse model.

Materials and Methods

Animals

This experimental study was carried out on 18 healthy adult sexually mature male Naval Medical Research Institute (NMRI) mice (7 weeks of age, 20±3 g body weight). Animals were obtained from the Animal House of Faculty of Veterinary Medicine, Urmia University, Urmia, Iran and housed in filter-top polycarbonate cages in an air-conditioned room (temperature: 25±2 ˚C, relative humidity: 50±10%, and 12 h light/12 h dark photoperiod) free from any sources of chemical contamination with free access to standard diet and water throughout the experimental period. Surgical procedure was conducted under cocktail intraperitoneally injection anaesthesia that the cocktail consisted of 0.1 ml xylazineand 1 ml ketamine and 8.9 ml distilled water at a dose of 0.1 ml/10 g BW [11]. The experimental protocol and procedures were carried out in accordance with international guidelines for care and use of laboratory animals and approved by the ethical committee of Urmia University.

Experimental Design

Following an adaptation period of seven days, mice were randomly divided into three groups of six mice each; control group, control-sham group and UIT group. In control group, mice only received ceftriaxone at a dose of 100 mg/kg BW intraperitoneally at the day of experimental UIT induction. Control-sham animals were anesthetized with cocktail (0.1 ml/10 g BW, IP) and only had exposure of the left vas deferens through a groin incision. During operation, mice received ceftriaxone at a dose of 100 mg/kg BW intraperitoneally. In UIT group, mice were anesthetized with cocktail (0.1 ml/10 g BW, IP) and UIT was induced by clamping left vas deferens with a mosquito clamp in fully locked fashion for 2 minutes. Then, the inguinal contents were replaced and the abdominal incision was tagged in 2 layers; 4-zero absorbable silk sutures were used for the musculature and skin. Ceftriaxone at a dose of 100 mg/kg BW was administered intraperitoneally to mice during operation. All groups were housed for 35 days in standard condition. At the end of treatment period, all animals in three groups were sacrificed with cervical dislocation after anesthetizing with cocktail.

Epididymal Sperm Analysis

Caudal epididymal sperms were collected by slicing the caudal region of the epididymis into small pieces in 1 ml of human tubal fluid (HTF) + 4mg/ml bovine serum albumin (BSA) and incubated for 10 minutes at 37 ºC in an atmosphere of 5% CO_2 to allow sperms to swim out of the epididymal tubules. The epididymal sperm count was obtained by the standard hemocytometric method. After dilution of epididymal sperm to 1:20 in HTF medium, approximately 10 µl of this diluted specimen was transferred to each of the counting chambers of the hemocytometer, which was allowed to stand for 5 minutes in a humid chamber to prevent drying. The cells sedimented during this time and were counted with a light microscope at 400×. The sperm count was expressed as number of sperm per milliliter [12].

The percentage of sperm motility was assessed visually using a light microscope at 400× magnification. For this procedure, one drop of sperm suspension was placed on a microscope slide and a cover slip was placed over the droplet. At least 10 microscopic fields were observed and motile sperms percentages were calculated [13].

In order to assess sperm viability, 20 µl of sperm suspension was mixed with 20 µl of 0.05% eosin-Y. After 2 minutes of incubation at room temperature, slides were viewed with magnification of 400 x. Sperm with altered plasma membrane seemed to be pink and sperm with intact plasma membrane were not stained. In each sample, 200 sperm cells were counted and the percentages of sperm viability (ratio of sperm with intact/altered plasma membrane) were recorded [14].

For evaluation of sperm abnormalities, sperm smears were prepared on clean and grease free slides, left to air-dry overnight, stained with 1% eosin-Y/5% nigrosin and examined at 400x magnification by light microscope. 200 sperm cells per animal were analysed to determine the percentage of sperms with

morphological abnormalitiessuch as amorphous, hook less, bicephalic, coiled or abnormal tails and attached cytoplasmic droplet [15].

Acridine orange (AO) test is a made easier microscopic method of the sperm chromatin structure assay which reflects sperm chromatin denaturation. A drop of the sperm suspension was spread on the glass slides and allowed to air-dry. All the smears were fixed for 2 hours in (methanol-acetic acid, 1:3, v/v). The slides were then stained with 2–3 cc 19% AO solution in phosphate citrate for 5 min and washed with deionized water. 200 sperms were evaluated with a fluorescence microscope and sperm heads with intact chromatin had green fluorescence, while those with denatured chromatin had orange-red staining [14].

In Vitro Evaluation of Epididymal Sperm Fertilizing Capacity

Four-week-old female mice were superovulated by intraperitoneal injection of 7.5 IU pregnant mare's serum gonadotropin (PMSG, Folligon, Netherlands) followed by intraperitoneal injection of 7.5 IU human chorionic gonadotropin (hCG, Folligon, Netherlands) 48 hours later [16]. 14 hours after hCG injection, female mice were anesthetized with cocktail (0.1 ml/10 gBW) and euthanized by cervical dislocation. Their oviducts were dissected and transferred to petri dishes that contained HTF medium.Using a stereo zoom microscope, ovulated oocytes were collected from the ampullary portions of the oviducts by dissecting technique. All oocytes after washing with HTF medium were transferred into the fertilization medium [17].

One day before conception, required media were prepared for fertilization and were incubated for 12 hours in a gas mixture of 5% CO_2 at 37 ˚C. For each of the groups, separate dishes were considered. Conception dishes with HTF medium which were combined with 4 mg/ml BSA were drop-marked. A drop of 500 ml and a few drops of 100 ml per dish were used for washing the *in vitro* fertilization (IVF) dishes and they were covered with mineral oil. Ten to 15 µl of capacitated sperm suspension was added to the fertilization medium to give the final motile sperm concentration of 2×10^6/ml. About 6 hours after adding sperms, fertilization determined by observation of male and female pronuclei under inverted microscope. Fertilized oocytes were removed from the fertilization drops, and rinsed 3 times with HTF medium. They were further cultured in 50 µl of fresh HTF medium+4mg/ml BSAfresh medium that had been pre-equilibrated and covered with mineral oil at 37 ˚C in 5% CO_2 and 95% humidity. Two-cell and blastocyst stage embryos percentages were evaluated 24 hours and 4-5 days after fertilization, respectively [18].

Statistical Analysis

All data were expressed as the mean ± standard error of mean (S.E.M.). The variables were analyzed by one-way analysis of variance followed by Tukey test for post hoc comparisons using Statistical Package for the Social Sciences, version 18.0, SPSS Inc, Chicago, Illinois, USA. The statistical significance level was set at $p<0.05$.

Results

Epididymal Sperm Parameters

Table 1 exhibits the effects of different treatments on epididymal sperm characteristics. As it is clear from the table, UIT caused a significant decrease in the both epididymal sperm concentrations compared to the control and control-sham groups. Also, comparison of the average number of epididymal sperms in UIT group showed a significant decrease in left epididymal sperms compared to the right. Significant differences were not observed in other groups (Table 1).

UIT caused a significant decrease in the left and right epididymidal sperms motility compared to the control and control-sham groups. However, significant differences were not observed following comparison of the left and right epididymidal sperms motility in UIT group. Also, significant differences were not found in other groups (Table 1).

The percentage of sperm viability in both epididymides of UIT group was significantly lower than both epididymides of control and control-sham groups (Figure 1). Also, the percentage of left epididymis sperms viability was significantly lower than rightepididymis in UIT group (Table 1).

Table 1. Bilateral effects of unilateral iatrogenic vas deferens trauma onepididymal sperm parameters.

Experimental Groups	Sperm count (10^6/mL)	Sperm motility (%)	Sperm viability (%)	Abnormal sperms (%)	AO[a]-positive sperms (%)
Control					
Ipsilateral epididymis	12.80±0.50 [a]	87.30±0.89 [a]	90.00±0.57 [a]	12.66±0.88 [a]	4.66±0.88 [a]
Contralateral epididymis	12.93±0.17 [a]	85.96±0.58 [a]	87.66±0.88 [a]	13.00±1.73 [a]	3.66±0.88 [a]
Control-sham					
Ipsilateral epididymis	12.73±0.58 [a]	80.23±4.32 [a]	85.33±0.88 [a]	34.00±0.57 [b]	9.33±0.88 [a]
Contralateral epididymis	12.76±0.76 [a]	80.63±4.06 [a]	85.00±2.51 [a]	32.33±0.66 [b]	7.00±0.57 [a]
UIT[b]					
Ipsilateral epididymis	4.10±0.35 [b]	51.93±0.26 [b]	56.33±1.45 [b]	42.33±1.20 [c]	51.00±1.15 [b]
Contralateral epididymis	6.91±0.45 [c]	60.93±0.37 [b]	63.66±0.88 [c]	50.33±1.20 [d]	36.33±2.60 [c]

[a]AO: Acridine orange; [b]UIT: Unilateral Iatrogenic vas deferens Trauma (Different superscripts denote statistical difference at a $p<0.05$).

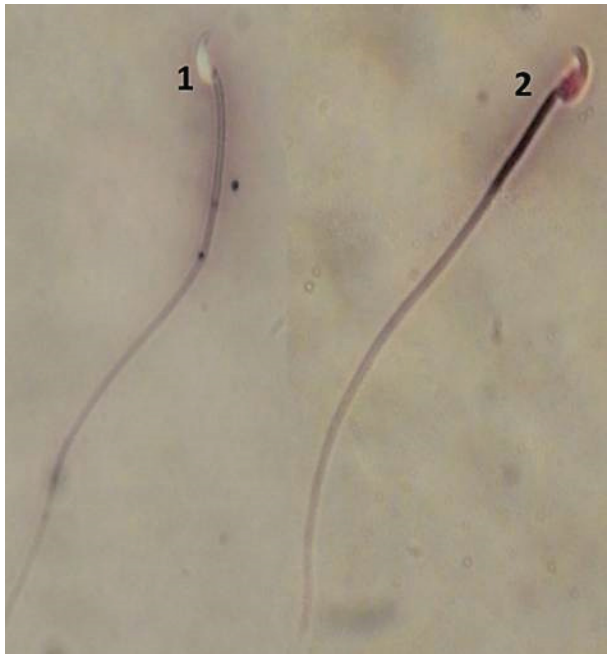

Fig. 1. Photomicrograph of epididymal sperms stained with eosin-Y in UIT group: Dead sperm (2) appear pink and live sperm (1) is not stained (×1000).

The percentage of sperm abnormality was increased significantly in both epididymides of UIT group when compared to control and control-sham mice. Also, percentage of sperm abnormality in the left epididymis of UIT group was significantly higherthan right epididymis. Interestingly, mean percentage of abnormal spermsin both epididymides of control-sham animals was significantly higher than those of the control mice (Table 1).

The results of AO staining revealed that the mean percentage of spermatozoa with DNA damage increased significantly in both epididymides of UIT group when compared to control and control-sham mice. Moreover, the mean percentage of spermatozoa with DNA damagein the left epididymis of UIT group was significantly higher than right epididymis. Significant differences were not observed in other groups (Table 1).

IVF Outcomes

Table 2 represents data concerningepididymal sperm *in vitro* fertilizing capacity in all experimental groups. UIT caused a significant decrease in the both epididymides sperms fertilization rate compared to the control and control-sham groups. Comparison of fertilization rates between left and right epididymides in the UIT group did not reveal significant differences. Significant differences were not seen in other groups (Table 2).

The percentage of two-cell embryos from left epididymis sperms of the UIT group showed a significant decline in comparison with both epididymides of control and control-sham groups. However, comparison of two-cell embryo percentages between left and right epididymides of UIT group did not reveal significant differences (Table 2).

UIT caused a significant decrease in blastocyst formation of both epididymides sperms in UIT group compared to the control and control-sham groups. Further, percentage of blastocyst stage embryos from left epididymis of UIT group was significantly reduced in comparison with rightepididymis (Table 2).

The percentage of hatched blastocysts from both epididymides sperms of UIT group markedly declined in comparison with both epididymides of control and control-sham groups. No statistical significance was foundin other groups (Table 2).

Discussion

The findings of present experimental animal study showed that UIT has deleterious bilateral effects on epididymal sperm characteristics and *in vitro* fertilizing capacity in mice. To the best of our knowledge, this study is unique in the literature in the sense thatthe relationship between UIT and male fertility was evaluated in an *in vitro* fertilization model.

Some infant surgeries accomplish due to inguinal region pathology. The vas deferens and vessels can be inadvertently manipulated over the surgical procedures. Accidental injuries and their effects in the inguinal region are inevitable and unpredictable and iatrogenic lesions may impair the performance of the vas deferens for long periods. In this type of injuries, even a slight injury to the muscular layers or mucous layer can affect fertility [6].

Inguinal hernia repair is one of the most common surgeries for pediatric and/or general surgeons[4]

Table 2. Bilateral effects of unilateral iatrogenic vas deferens trauma on epididymal sperm *in vitro* fertilizing capacity.

Experimental Groups	Oocytes	Fertilization rate (%)	Two-cell embryos (%)	Blastocysts (%)	Hatched blastocysts (%)
Control					
Ipsilateral epididymis	114	(107) 93.79±1.20^{a}	(98) 91.49±1.49^{a}	(90) 85.10±0.89^{a}	(82) 76.71±1.28^{a}
Contralateral epididymis	119	(115) 96.85±0.91^{a}	(102) 87.81±3.72^{a}	(97) 82.41±2.05^{a}	(86) 73.95±3.50^{a}
Sham					
Ipsilateral epididymis	111	(104) 93.63±1.18^{a}	(91) 87.17±5.54^{a}	(86) 82.41±4.86^{a}	(73) 69.81±6.55^{a}
Contralateral epididymis	116	(109) 93.99±0.45^{a}	(98) 89.92±0.27^{a}	(92) 84.51±1.76^{a}	(77) 70.51±1.89^{a}
UIT[a]					
Ipsilateral epididymis	124	(64) 51.52±0.58^{b}	(40) 63.05±3.60^{b}	(23) 35.58±2.25^{b}	(8) 12.31±1.20^{b}
Contralateral epididymis	112	(78) 69.83±1.58^{c}	(57) 73.18±1.09^{ab}	(41) 52.72±1.56^{c}	(11) 14.38±2.76^{b}

[a]UIT: unilateral iatrogenic vas deferens trauma. (Different superscripts denote statistical difference at a $p<0.05$).

and vas deferens injuries are serious complications of inguinal herniotherapy. Manipulations during surgeries and mesh-related fibrosis can result in perforation or obstruction of injured vas deferens leading tomale impaired fertility [19]. Previous studies have pointed to the consequences of the vas deferens surgical manipulations such as: grasping with toothed and non-toothed forceps, clamping with mosquito haemostats and electrocoagulation that can lead to histological alterations including intra- and transmural inflammatory reaction, mural and muscular wall disruptions and luminal obliteration [4].

It is well documented that vas deferens obstruction causes testicular blood flow reduction and eventually testicular hypoxia and atrophy [20,21]. The exact mechanism responsible for unilateral vassal injury-mediated bilateral testicular damages have not been fully elucidated yet, but antisperm autoantibodies (ASAs) formation and sympathetic vasospasm may contribute [10,21]. It has been established that spermatozoa out flow obstruction can result in ASAsformation leading to sperm motility and fertilizing capacity reduction through either agglutination or immobilization [10]. It was also found that ASAs affect sperm-oocyte interaction by impairing sperm capacitation and acrosomal reaction [22,23]. Moreover, extensive researches demonstrate that sympathetic nervous system plays a critical role in vas deferens obstruction related injuries through reflexive reduction of contralateral blood flow leading to testicular degeneration [21,24].Consistent with above-noted findings, UIT in this studycaused significant decreases in the sperm concentration, motility, viability and fertilizing capacity as well as elevated sperm DNA damage. Additionally, our findings are confirmed byprevious studies that reported vas deferens injuries cause azoospermia [17,25].

It is known that epididymis plays important roles in sperm storage and maturation and long term storage of sperms in epididymis leads to agingand impaired sperms motility along with decreased fertilization rates [26,27]. Furthermore, vas deferens absorptive and secretory functions are involved in formation of supportive environment for sperm fertility potential development [28].Supporting these facts, bilateral reduction of IVF rate following UIT may be attributed to the sperm maturation procedure impairmentand subsequently declined sperm fertilizing capacity. Consistent with our findings, prior studies have pointed out thathigh pressure within the epididymis and seminifer ous tubules due to vas deferens obstruction results in testicular fibrosis and sperm destruction leading to lower IVF outcome [17].

In summary, the present study provided evidence that UIT has detrimental bilateral effects on epididymal sperm characteristics and *in vitro* fertilizing capacity in mice. It is, therefore, recommended that the most caution should be considered in inguinal region surgeries to avoid vas deferens injury.

Acknowledgments

This study was supported by a grant from Urmia University.

Conflict of Interest: None declared.

References

1. Kohn LT, Corrigan JM, Donaldson MS. To err is Human: Building a Safer Heath System. Washington: National Academies Press; 2000. p. 49-68.
2. Antypas S, Sofikitis N, Rodriguez F, Zavos PM, Miyagawa I. Bilateral effect of unilateral vasectomy on testicular testosterone biosynthesis. *J Pediatr Surg*. 1994;**29**(6):828-31.
3. Janik JS, Shandling B. The vulnerability of the vas deferens (II): the case against routine bilateral inguinal exploration. *J Pediatr Surg*. 1982;**17**(5):585-8.
4. Blouchos K, Boulas K, Tselios D, Hatzigeorgiadis A, Kirtsis P. Iatrogenic vas deferens injury due to inguinal hernia repair. *Hellenic Journal of Surgery*. 2012;**84**(6):356-63.
5. Schlegel PN. Causes of azoospermia and their management. *Reprod Fertil Dev*. 2004;**16**(5):561-72.
6. Abasiyanik A, Guvenc H, Yavuzer D, Peker O, Ince U. The effect of iatrogenic vas deferens injury on fertility in an experimental rat model. *J Pediatr Surg*. 1997;**32**(8):1144-6.
7. Shandling B, Janik JS. The vulnerability of the vas deferens. *J Pediatr Surg*. 1981;**16**(4):461-4.
8. Matsuda T, Horii Y, Yoshida O. Unilateral obstruction of the vas deferens caused by childhood inguinal herniorrhaphy in male infertility patients. *Fertil Steril*. 1992;**58**(3):609-13.
9. Chakraborty J, Hikim AP, Jhunjhunwala JS. Quantitative evaluation of testicular biopsies from men with unilateral torsion of spermatic cord. *Urology*. 1985;**25**(2):145-50.
10. Chehval MJ, Martin SA, Alexander NJ, Winkelmann T. The effect of unilateral injury to the vas deferens on the contralateral testis in immature and adult rats. *J Urol*. 1995;**153**(4):1313-5.
11. Hoff JB, Dysko R, Kurachi S, Kurachi K. Technique for performance and evaluation of parapharyngeal hypophysectomy in mice. *J Am Assoc Lab Anim Sci*. 2006;**45**(2):57-62.
12. Jalali AS, Hasanzadeh S, Malekinejad H. *Achillea millefolium* inflorescence aqueous extract ameliorates cyclophosphamide-induced toxicity in rat testis: stereological evidences. *Chinese Journal of Natural Medicines*. 2012;**10**(4):247-54.
13. Selvakumar E, Prahalathan C, Sudharsan PT, Varalakshmi P. Chemoprotective effect of lipoic acid against cyclophosphamide-induced changes in the rat sperm. *Toxicology*. 2006;**217**(1):71-8.
14. Zahra A, Gholamreza N, Farokhi F, Shalizar Jalali A. Attenuation of Cyclosporine-Induced Sperm Impairment and Embryotoxicity by Crataegus monogyna Fruit Aqueous Extract. *Cell J*. 2013;**15**(3):198-205.
15. Shalizar Jalali A, Hasanzadeh S, Malekinejad H. Chemoprotective effect of *Crataegus monogyna* aqueous extract against cyclophosphamide-induced reproductive toxicity. *Vet Res Forum*. 2011;**2**:266-273.
16. Byers SL, Payson SJ, Taft RA.

Performance of ten inbred mouse strains following assisted reproductive technologies (ARTs). *Theriogenology*. 2006;**65**(9):1716-26.

17. Ikeda T, Sofikitis N. Bilateral testicular consequences in the unilateral vasectomy of immature rats. *Yonago Acta Medica*. 2000;**43**:1-9.

18. Cebral E, Carrasco I, Vantman D, Smith R. Preimplantation embryotoxicity after mouse embryo exposition to reactive oxygen species. *Biocell*. 2007;**31**(1):51-9.

19. Ridgway PF, Shah J, Darzi AW. Male genital tract injuries after contemporary inguinal hernia repair. *BJU Int*. 2002;**90**(3):272-6.

20. Whyte J, Sarrat R, Cisneros AI, Whyte A, Mazo R, Torres A, et al. The vasectomized testis. *Int Surg*. 2000;**85**(2):167-74.

21. Pampal A, Ozen IO, Ekingen G, Demirogullari B, Helvacioglu F, Take G. The morphological evaluation of ipsilateral and contralateral vasa deferentia in a rat model of unilateral spermatic cord torsion. *Pediatr Surg Int*. 2010;**26**(3):287-92.

22. Hendry WF, Hughes L, Scammell G, Pryor JP, Hargreave TB. Comparison of prednisolone and placebo in subfertile men with antibodies to spermatozoa. *Lancet*. 1990;**335**(8681):85-8.

23. Ozturk U, Ozdemir E, Dede O, Sagnak L, Goktug HN, Gurbuz OA, et al. Assessment of anti-sperm antibodies in couples after testicular sperm extraction. *Clin Invest Med*. 2011;**34**(3):E179-83.

24. Kutlu O, Kocabiyik A, Koksal IT, Guntekin E. Effects of chemical sympathectomy on contralateral testicular histology and fertility in unilateral vasectomy. *J Korean Med Sci*. 2009;**24**(5):849-52.

25. Sandhu DP, Osborn DE, Munson KW. Relationship of azoospermia to inguinal surgery. *Int J Androl*. 1992;**15**(6):504-6.

26. Zenzes MT, Reed TE, Nieschlag E. Non-poisson distribution of sperm from grandfathers in zona-free hamster ova. *J Androl*. 1991;**12**(1):71-5.

27. Yeung CH, Cooper TG, Oberpenning F, Schulze H, Nieschlag E. Changes in movement characteristics of human spermatozoa along the length of the epididymis. *Biol Reprod*. 1993;**49**(2):274-80.

28. Bedford JM. The status and the state of the human epididymis. *Hum Reprod*. 1994;**9**(11):2187-99.