Recruiting Testicular Torsion Introduces an Azoospermic Mouse Model for Spermatogonial Stem Cell Transplantation

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Received January 2013
Accepted June 2013

Purpose: To investigate the long-term effect of testicular torsion on sperm parameters and testis structure in order to introduce a novel mice azoospermic model for spermatogonial stem cell transplantation.

Materials and Methods: Unilateral testicular torsion was created. The animals were divided into two groups each containing 15 mice. They underwent 2 and 4 hours of unilateral testicular ischemia, respectively. All animals in this experiment were aged matched. The experimental ($n = 5$) groups were studied 2, 4 and 10 weeks after testicular ischemia reperfusion. Moreover, the left testes and epididymis were removed for sperm analysis and for weight and histopathological evaluation. Finally isolated spermatogonial stem cells were transplanted in the testes that underwent 2 hours of ischemia reperfusion, two weeks post-surgery.

Results: All the investigated parameters demonstrated a sharp decline at 2, 4 and 10 weeks after testicular torsion, whereas 2-hour ischemia was found to be less injurious in testicular tissue structure. Two months after xenotransplantation, the transplanted cells were localized in the basal of the seminiferous tubules of the recipient ischemic testes.

Conclusion: Torsion can cause permanent azoospermia in mouse. Also Testicular torsion 2 weeks after the 2 hours ischemia reperfusion may prove useful for recipient preparation for SSCs transplantation in mouse.

Keywords: adult stem cells; transplantation; animals; fertility preservation; mice; testis; spermatogonia; reperfusion injury; blood supply.

INTRODUCTION

Spermatogonial stem cells (SSCs) transplantation is proven to be an efficient technique for studying Sertoli cells and germ cells interactions via the model preparation. This promising technology can also provide transgenic animals.[1] In addition, SSC transplantation has been widely used to investigate the spermatogenesis recovery in various species.[2-4] The first critical step in successful transplantation is preparation of the recipient testes.[5] Hence, depletion of internal germ cells with minimal damage to the local spermatogenic microenvironment and stem cell niches are requisite.[5] On the other hand, the SSCs fate is controlled by factors associated with stem cell niche, which reside near the basement membrane of seminiferous tubules in the vicinity of Sertoli cells. However, an assessment of stem cell niche alterations remains difficult in order to evaluate the present SSCs recipient model, so the only way in which stem cell activity and microenvironment can be determined is via a transplantation assay.[6] Different methods have been suggested to deplete testicular germ cells from immature and adult animals[5,7] including treatment with cytotoxic agents. These may consist of busulfan[8] or radiation[9], induction of cryptorchidism[10], hyper- or hypothermia[11] and vitamin A deficiency.[12] Most of these approaches can effectively deplete the majority of SSC, however damaging side effects and prolonged duration of treatment are the main disadvantages of these methods.[13] Testicular torsion is a urological emergency disorder in which one testicle gets twisted in the scrotum, subsequently cutting off its blood supply. An affected testicle tends toward ischemia and reproductive system dysfunction.[14] Although the germ cell apoptosis was reported following testicular torsion, long term histopathologic changes of the testes following the torsion have not been studied yet.[15]

Long-term investigation of testicular torsion effects on sperm parameters and testis structure and also assessment of stem cell niche alterations were the main aims of the present study. It is believed that the present model is a proper tool in SSC transplantation for treating testicular torsion complications and for performing further medical researches on infertility and spermatogenesis.

MATERIALS AND METHODS

Animal Preparation and Spermatic Cord Torsion

A total of 70 male NMRI mice at 6-8 weeks of age were purchased from Razi Vaccine and Serum Research Institute (Karaj, Iran). Animals were divided into two groups each containing 15 mice. In the first and second groups, animals underwent two and four hours of unilateral testicular ischemia (via a 720° torsion in a counterclockwise direction, fixed to the scrotum with a 6-0 nylon suture passing through the tunica albuginea and dartos,[16] respectively. After proper time of ischemia, the suture was removed and the left testis was untwisted and replaced in the scrotum and the incision was closed. Control (n = 15) and sham (n = 15) animals were matched to the ages of the experimental groups. Sham surgeries involved a midline scrotal incision and physical manipulation of the testis before placing it back into the scrotum. The mice (n = 5) were sacrificed by cervical dislocation 2, 4 and 10 weeks after testicular ischemia reperfusion. The left testes and epididymis were removed for sperm analysis and for weight and histopathological evaluation. The mice were fed with standard commercial laboratory chow [(pellet form), Javeneh Khorasan Co., Mashhad, Iran], water and housed under standard laboratory conditions (12 h light: 12 h dark at 22 ± 2˚C) during the experimental period. All animal experiments were aged matched and approved by the Animal Ethics Committee at Tehran University of Medical Sciences. All operations were performed under sterile condition with the subject under 100 mg/kg of ketamine hydrochloride (Rotexmedica, Trittau, Germany) and 10 mg/kg xylazine (Alfasan, Holland) anesthesia (single intraperitoneally).

Histopathologic Evaluation

The testes of mice were removed, weighed and fixed in a Bouin's mixture, embedded in paraffin, sectioned at 5 µm thicknesses and finally stained with hematoxylin and eosin (H&E). One hundred seminiferous tubules that were round or nearly round for each testis were assessed randomly. The mean seminiferous tubule diameter and epithelium height was measured in each testes using Image J (Version 1.240; National Institutes of Health, USA). The seminiferous tubules were graded according to the Johnsen score system in which seminiferous tubules in each section are evaluated systematically and each is given a score.[17]

Sperm Quality Parameters

Left cauda epididymis and vas deferens were isolated and placed in 1 mL of phosphate buffered saline (PBS) (pH = 7.4), prior to be minced using sharp scissors. The spermatozoa were allowed to swim out for 15 min in an atmosphere of 5% CO2 at 37˚C, for the purpose of sperm quality and motility analyzed under light microscope (Olympus, type CH2, 400 × magni

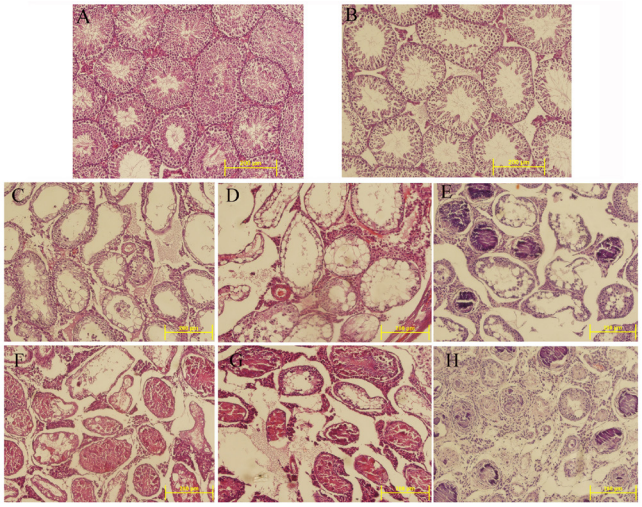

Figure 1. Figure 1. Comparison of seminiferous tubule deterioration in different groups of our study following torsion. Normal seminiferous tubules are seen in control (A), sham (B) and treated groups during long time. Effects of 2-hour ischemia after 2 weeks (C), 4 weeks (D) and 10 weeks (E), also injurious and irreversible effects of a 4-hour testicular torsion was observed at 2 weeks (F), 4 weeks (G) and 10 weeks (H) post-surgery (Bar = 200 µm).

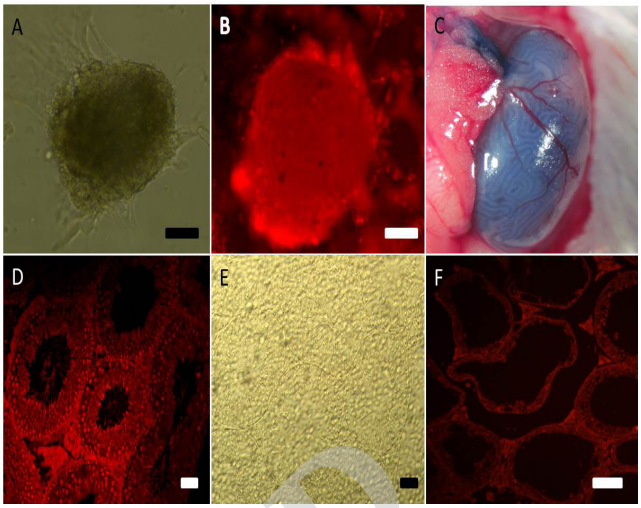

Figure 2. Cell labeling of spermatogonial stem cell and transplantation. (A) cluster of spermatogonial stem cells, (B) labeled cell with DiI, (C) the transplanted spermatogonial cells, (D) colonization and proliferation of labeled injected cells, (E) unstained seminiferous tubule, and (F) section of testis 10 weeks after testicular torsion under ultraviolet light without any obvious signals (Bar = 50 µm).

fications) The sperm suspensions were counted using Neubauer counting chamber (Thoma, assistant Sondheim/Rhon, Germany) for determination of sperm concentration and expressed as ×106/mL. Eosin B (0.5% in saline) was used to determine the percentage of viable sperm (sperm with red head was counted as dead sperm).

SSCs Isolation and Culture

Bilateral testes from ten neonate 3-6-day-old NMRI mice were gathered for SSC isolation. Testis cells were obtained by two-step enzymatic digestion and used for culture utilizing the method previously described with some modifications.[18] In brief, minced testis pieces were suspended in DMEM containing collagenase and trypsin 1 mg/mL each (both from Gibco, Paisley, Scotland, UK), as well as hyaluronidase type II 1 mg/mL (Sigma) and incubated at 37˚C for 15 min with shaking and a little pipetting. After removal of interstitial cells by 3 washes in dulbecco's modified eagle's medium (DMEM), seminiferous cord fragments were incubated in DMEM after adding fresh enzymes for 30-45 min as described above. Cells were separated from the remaining tubule fragments by centrifugation at 300g (1200 rpm) for 5 min. After filtration through sterile mesh (41-µm opening, 5-6 cm^2), the cells were pelleted and subjected to differential plating to eliminate the somatic cells (myoid and Sertoli cells).[19,20] The obtained cells were cultured at 37°C and 5% CO2, in a humidified atmosphere in the presence of 2.5% fetal bovine serum (FBS) and glial cell line-derived neurotrophic factor (GDNF) 10 ng/mL for two weeks.

Transplantation Procedure and Assay

The spermatogonial-cell-derived colonies were labeled with DiI (Invitrogen, Carlsbad, CA, USA) based on manufacturer's protocol and transplanted into the seminiferous tubules of the recipient mice that had undergone two hours of left testicular ischemia 2 weeks after reperfusion via rete testis. The recipient mice (n = 10) were anesthetized as described. Approximately, 105 cultured SSCs in 10 µL DMEM were then injected into the seminiferous tubules in affected testis of each recipient mouse. Transplantation was performed by retrograde injection through the efferent ducts,[21] with some modifications. Seminiferous tubules were tracked and visualized by adding trypan blue in the germ cell injection media. The existence and proliferation of injected cells were evaluated 10 weeks post transplantation under fluorescent microscope (Olympus, type CH2, 400 × magnification).

Statistical Analysis

Variables were presented as means (±SD) and the normal distribution of data was evaluated and then confirmed by Kolmogorov–Smirnov test. To assess the effect of interventions,

Table 1. Spermatogenesis evaluation during different time after surgery by assessment of Johnsen score, mean epididymis sperm count, percentage of sperm motility, percentage of viable sperm.*

		Duration		
	Groups	**2 Weeks**	**4 Weeks**	**10 Weeks**
Johnsen score	Control	9.34 ± 0.46	9.36 ± 0.47	9.08 ± 0.51
	Sham	9.20 ± 0.87	9.10 ± 0.55	9.24 ± 0.61
	Torsion 2 hours	2.60 ± 0.12[a]	1.52 ± 0.63[a, c]	2.46 ± 0.82[a]
	Torsion 4 hours	1.36 ± 0.29[a, b]	1.20 ± 0.12 [a]	0.00 ± 0.00[a, b, c, d]
Sperm count (Mean±SD) ×106	Control	6.12 ± 0.92	6.30 ± 0.84	5.96 ± 0.79
	Sham	5.98 ± 0.94	6.48 ± 1.39	6.00 ± 0.91
	Torsion 2 hours	0.19 ± 0.16[a]	0.00±0.00[a]	0.01 ± 0.01[a]
	Torsion 4 hours	0.01 ± 0.01[a]	0.00 ±0.00[a]	0.00 ± 0.00[a]
Sperm motility (%)	Control	60.60 ± 7.44	61.00 ± 6.36	60.00 ± 7.11
	Sham	61.20 ± 14.08	63.00 ± 12.0	60.60 ± 13.43
	Torsion 2 hours	1.80 ± 1.92[a]	0.00 ± 0.00[a]	0.60 ± 0.89[a]
	Torsion 4 hours	0.00 ± 0.00 [a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Sperm viability (%)	Control	67.00 ± 7.21	65.00 ±7.18	65.20 ± 7.05
	Sham	71.40 ± 7.40	70.20 ± 7.12	72.00 ± 4.12
	Torsion 2 hours	3.00 ± 2.12[a]	0.0 ± 0.00[a]	1.80 ± 2.49[a]
	Torsion 4 hours	0.60 ± 0.89 [a]	0.00 ± 0.0[a]	0.00 ± 0.0[a]

* The results of five separate experiments were used for all groups. The values are the mean ± SD at different times.

[a] Significant difference versus control and sham in the same column ($P \leq .05$).

[b] Significant difference versus torsion 2 hours in the same column ($P < .05$, Based on Tukey post hoc test).

[c] Significant difference versus 2 weeks in the same row ($P \leq .05$).

[d] Significant difference versus 4 weeks in the same row ($P < .05$, Based on Sidak post hoc test).

time and possible interaction of these effects, mixed model analysis was applied by choosing diagonal covariance structure based on minimum Akaike Information criterion (AIC). For post hoc tests two series of analyses were performed. To investigate the effect of intervention and comparing groups in various time points before and after intervention, analysis of variance (ANOVA) and covariance (ANCOVA) were used, respectively. In post-intervention group, an adjustment for baseline measurements followed by Sidak post hoc test in most cases was performed (the false positive rate was controlled and remained on 0.05 level). To compare time points in each group, mixed model analysis was used by choosing diagonal covariance structure followed by Sidak post hoc test in significant cases. All analyses were performed using statistical package for the social science (SPSS Inc, Chicago, Illinois, USA) version 18.0 at a .05 significance level.

RESULTS

Sperm Analysis Following Testicular Torsion

Based on the sperm analysis parameters in sham and control animals no considerable discrepancy was identified throughout 10 weeks of evaluations. However, a significant reduction in epididymal sperm count was perceived 2 weeks following testicular ischemia reperfusion and vanished 4 weeks after treatment compared to sham and control groups ($P = .001$ and $P = .015$, respectively). Whereas no significant difference was observed for 2 and 4 hours treated animals. Also different values obtained during 2, 4 and 10 weeks of our assessment were not significant (Table 1).

There is a significant decline in sperm motility 2 and 4 hours after testicular torsion, and two weeks post-surgery ($P = .001$ and $P = .009$, respectively). However, no significant difference was found between experiment mice including 2, 4 and 10 weeks post-surgery as well as 2 and 4 hours of ischemia (Table 1). Furthermore, sperm viability reduction was observed in both groups that underwent 2 and 4 hours of ischemia, 2, 4 and 10 weeks after surgery ($P = .001$, $P = .05$, $P = .033$, respectively). Neither there was any significant difference of

Table 2. Histopathologic evaluation of testicular tissue by weight, seminiferous tubule diameter and seminiferous epithelium diameter.*

	Groups	2 Weeks	4 Weeks	10 Weeks
Testicular weight (gr)	Control	0.134 ± 0.011	0.130 ± 0.019	0.124 ± 0.011
	Sham	0.123 ± 0.008	0.118 ± 0.013	0.119 ± 0.009
	Torsion 2 hours	0.078 ± 0.007[a]	0.064 ± 0.012[a]	0.068 ± 0.015[a]
	Torsion 4 hours	0.073 ± 0.012[a]	0.063 ± 0.013[a]	0.066 ± 0.024[a]
Seminiferous tubule	Control	200.00 ± 5.34	198.60 ± 4.13	201.18 ± 4.12
	Sham	199.28 ± 4.01	198.48 ± 2.55	198.82 ± 1.17
	Torsion 2 hours	143.24 ± 9.17 [a]	148.00 ± 16.44 [a]	136.46 ± 8.87[a]
	Torsion 4 hours	126.92 ± 5.66[a, b]	126.40 ± 3.39[a, b]	109.76 ± 3.16 [a,b,c]
Seminiferous epithelium	Control	58.84 ± 12.19	63.34 ± 11.51	62.22 ± 11.28
	Sham	48.06 ± 8.03	47.82 ± 9.86	50.56 ±7.40
	Torsion 2 hours	18.94 ± 1.07[a]	10.04 ± 1.38 [a, c]	16.52 ± 6.77[a]
	Torsion 4 hours	5.10 ± 3.28[a, b]	6.28 ± 1.62[a]	2.62 ± 1.12[a b, d]

* The results of five separate experiments were used for all groups. The values are the mean ± SD at different times.

[a] Significant difference versus control and sham in the same column ($P \leq .05$).

[b] Significant difference versus torsion 2 hours in the same column ($P < .05$, Based on Tukey post hoc test).

[c] Significant difference versus 2 weeks in the same row ($P \leq .05$).

[d] Significant difference versus 4 weeks in the same row ($P < .05$, Based on Sidak post hoc test).

the ischemia duration effect on the sperm viability (Table 1).

Histopathological Observations

Figure 1 illustrates testes sections from animals used in this study (Control, Sham and experimental groups) under a light microscopy. Testis sections of Control and sham animals showed a normal seminiferous tubule contour and morphology (Figures 1 A and B). The epithelium of all seminiferous tubules in the treated groups was severely disrupted and most of tubules depleted 2 weeks post operation. In some tubules only the basement membrane was observed. However the 4 hours testicular ischemia showed a more serious and irreversible effect and the sclerotic seminiferous tubules were observed 10 weeks after the 4 hours ischemia and reperfusion (Figure 1). Despite the reversible effects of 2-hour ischemia on testicular tissue, damaging effects and persistent spermatogenesis arrest was observed up to 10 weeks post operation based on histopathological observation.

Assessment of Morphological Changes

Reduction in left testis weight was remarkable compared to that of sham and control groups ($P \leq .05$) 2 weeks after reperfusion of testicular torsion; however, testicular weight value was not correlated with the duration of ischemia (Table 2). As shown in Table 2, the tubule diameter was lesser significantly in 4-hour than 2-hour ischemia during 2, 4 and 10 weeks after surgery ($P = .001$). However in 4-hour-ischemia group, tubule diameter reached its minimum after 10 weeks (Table 2). Similar results were also obtained for thickness of the seminiferous germinal epithelium, with this exception that the significant reduction in epithelial thickness was observed in 2-hour group, two weeks after ischemia. Furthermore a non-significant increase in thickness of seminiferous epithelium and regeneration of seminiferous epithelium was observed for 2 hours group, 10 weeks following ischemia (Table 2).

Johnsen Score Assessment

Following torsion, the mean Johnsen score was dramatically declined at different time intervals during the study in comparison to sham and control groups ($P = .005$). Seminiferous tubule score for 4-hour testicular ischemia was significantly lower than that of 2-hour ischemia, two weeks following operation and it reached a score of zero, 10 weeks after ischemia ($P = .001$). Based on Johnsen scoring, most seminiferous tubules were depleted 2 weeks following ischemia and it persists for 10 weeks after ischemia-reperfusion (Table1).

Spermatogonial Stem Cell Culture and Transplantation

The clusters appeared in 2-3 days after primary culture. When these clusters were enzymatically dispersed and re-plated, their SSC contents could start new clusters during 2 weeks of culture (Figure 2). Cell labeling with DiI was performed

before transplantation. Two months after transplantation, florescent labeled spermatogonial stem cell considered as transplanted cells. The labeled cells were localized in the basal compartment of seminiferous tubules of the recipient testes. Two months after stem cell transplantation, colonization and proliferation of transplanted cells were found (Figure 2).

DISCUSSION

The results from this study showed that testicular torsion have extensive changes in mouse testis structure and epididymal sperms parameters upon 2 and 4 testicular ischemia reperfusions and the effects were irreversible up to 10 weeks. Preparation of SSCs transplantation in mouse case of testicular torsion 2 weeks after 2 hours ischemia reperfusion may also prove useful and applicable.

For preparation of the recipient testes in a successful transplantation, maximal depletion of endogenous germ cells with minimal defect to the local spermatogenic niche and microenvironment, are required.[22] Busulfan has been used to prepare transplantation recipients in a divers species, including mouse,[23] rat,[24] monkey,[25] and pig.[26] In other species, busulfan treated animal has not been well prepared for SSC transplantation. In rats, offspring have been obtained via germ cell transplantation in busulfan-treated recipients.[27,28] However, the efficiency of transplantation is poor due to dramatic disturb of the testicular SSC niche by busulfan. Testicular niche disturbance prevents transplanted SSCs differentiation and proliferation.[29] Busulfan administration can produce systemic toxicity and even lethality due to severe bone marrow depression.[26] The side effects of busulfan treatment limit the efficiency of SSC transplantation. Radiation is another method for recipient preparation, but the calcification of seminiferous tubules induced by irradiation, and this can impair the migration of transplanted cells.[7] A less harmful and more effective recipient model for SSC transplantation is desirable.

Testicular ischemia is the main consequence of testicular torsion, from both clinical and experimental points of view.[30] Since a severe disruption of seminiferous epithelium is observed during testes reperfusion just after torsion, the injury must occur either during the ischemia or upon reperfusion. Our data showed a remarkable decrease in epididymal sperm concentration following 2 and, 4 hours ischemia two weeks after surgery and reached zero 4 weeks post-intervention. Discrepancies in sperm count at 2nd, 4th and 10th week of our evaluation were non-significant. On the other hand, ipsilateral azoospermia occurred in epididymis of affected testis 2 weeks following reperfusion and persisted up to 10 weeks. Some long term studies have already been published on evaluating the absence of sperm in seminiferous tubule in histopathological observations following testicular torsion.[31,32] However, this study is the first to assess and evaluate sperm concentration following spermatic cord torsion, so far. Data revealed an intensive decline in Sperm motility and viability due to testicular torsion 2 weeks after ischemia and remained the same until the 10th week. Deterioration of testes structure does not seem to be the main cause of sperm parameters in testicular torsion 2 weeks post-surgery. The main stimulant components in pathophysiology of testicular torsion involve the generation of reactive oxygen species (ROS) after restoration of blood following the ischemia.[33] It has been demonstrated that ROS increased in the areas of ischemia and reperfusion, and is responsible for ischemia-reperfusion injury that has received little attention in the testis.[34,35] Spermatozoa are highly sensitive to oxidative stress, particularly to lipid peroxidation due to their high content of polyunsaturated fatty acids in the plasma membrane. A damaging role of ROS on ejaculated sperm was also clarified by a large number of studies.[36] Therefore, based on these results, the main cause of the poor sperm quality could be an increased ROS production. As mentioned, the implication of testicular torsion on epididymal sperm parameters has not already been identified. In general to be fertile, approximately 50% of the seminiferous tubules of a mouse must contain complete spermatogenesis.[37] Histopathological observations indicated that all the treated groups in this study are considered infertile, but after 4 hours of testicular torsion, a serious and irreversible injury was perceived. Histopathological reports of the previous studies were in agreement with our results.[31,32] Thus, it is not reasonable to assume a correlation for the SSC transplantation with sclerotic changes after 4 hours of testicular torsion due to disruption of seminiferous tubular structure deviation in the SSC niche and severe tubular ectasia.

Depletion of seminiferous tubules was shown by the testicular weight loss following testicular torsion. Moreover, similar results were perceived in other studies investigating the experimental torsion in the sham and control groups of animals. These studies demonstrated no effect on ipsilateral testis weight at several time points after torsion repair. Whereas, increasing the time of torsion to 1, 2, and 4 hours, caused a pro

gressive loss in testis weight, after 30 days from torsion repair and leaded to an almost total loss of spermatogenesis at both the 30th and 60th day.[30,38]

Seminiferous tubular diameter indicates the function of testis in spermatogenesis,[39] thus in our study significant reduction in spermatogenesis was detected following testicular ischemia. However, the amount of reduction depends on the duration of ischemia.[40] Although spermatogenesis defects by measuring seminiferous tubules epithelium and testicular tissue destruction following torsion was reported by several studies, the lack of accurate data on tissue structure is one of the drawbacks of these reports.[31,32,38]

The Johnsen score describes a new and rapid method for registration of spermatogenesis in testes. Pathognomonic score counts that lead to immediate diagnosis at a glance, were obtained in many instances. An obvious correlation has been found between sperm count and testicular biopsy scoring for the first time to correlate endocrine conditions with the functional state of the testicular tissue.[17]

The result of Johnsen score estimation was consistent with the other findings of our study. Intensive, irreversible injury was observed in damaged testis by 4-hour (but not by 2-hour) testicular ischemia. In addition, azoospermic mice were invented 2-week post 2-hour testicular torsion, so SCC was transplanted 2-week after the 2-hour testicular ischemia.

There are several applications for spermatogonial stem cell transplantation beyond basic researches.[41] Obtaining and storage of spermatogonial stem cells in special occasions including cancer treatment that may lead to infertility is beneficial. So providing cells from affected individuals for restoring fertility could be transplanted back into the patient, or at emergency situations that unilateral orchiectomy is indicated, a testicular biopsy would provide the spermatogonial stem cells for cryopreservation, storage, and transplantation.[41,42]

Standpoint of basic research, due to no specific biochemical or morphological markers for spermatogonial stem cells has not yet been determined. Therefore, the only assay for the presence of spermatogonial stem cells in a cell suspension is the spermatogonial stem cell transplantation technique.[5,7] SSC transplantation has been examined in various azoospermic animal models.[2,4,37,43]

CONCLUSION

In conclusion, the present study explains detailed information on the effects of testicular torsion on the testicular and epididymal parameters. Torsion can cause permanent azoospermia in mouse which is irreversible until 10 weeks. Also Testicular torsion might be useful in recipient preparation for SSCs transplantation in mouse, which might in turn lead to a novel treatment for infertility and other consequences of testicular torsion, clinically.

ACKNOWLEDGEMENTS

Authors would like to appreciate contribution and administrative support of professor MT Joghataei. This study was funded by a grant from Iran University of Medical Sciences, (Number: 90-04-30-14879) and all experiments have been performed at Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Honaramooz A, Yang Y. Recent advances in application of male germ cell transplantation in farm animals. Vet Med Int. 2010;2011.
2. Honaramooz A, Behboodi E, Blash S, Megee So, Dobrinski I. Germ cell transplantation in goats. Mol Reprod Dev. 2003;64:422-8.
3. Izadyar F, Den Ouden K, Stout Ta, et al. Autologous and homologous transplantation of bovine spermatogonial stem cells. Reproduction. 2003;126:765-74.
4. Koruji M, Movahedin M, Mowla SJ, Gourabi H, Pour-Beiranvand S, Jabbari Arfaee A. Autologous Transplantation of Adult Mice Spermatogonial Stem Cells into Gamma Irradiated Testes. Cell J. 2012;14: 82-9.
5. Brinster CJ, Ryu BY, Avarbock MR, Karagenc L, Brinster RL, Orwig KE. Restoration of fertility by germ cell transplantation requires effective recipient preparation. Biol Reprod. 2003;69:412-20.
6. Mclean DJ. Spermatogonial stem cell transplantation, testicular function, and restoration of male fertility in mice. Methods Mol Biol. 2008;450:149-62.
7. Zhang Z, Shao S, Meistrich Ml. Irradiated mouse testes efficiently support spermatogenesis derived from donor germ cells of mice and rats. J Androl. 2006;27:365-75.
8. Brinster Rl, Zimmermann JW. Spermatogenesis following male germ-cell transplantation. Proc Natl Acad Sci U S A. 1994;91:11298-302.
9. Koruji M, Movahedin M, Mowla SJ, Gourabi H, Arfaee AJ. The morphological changes of adult mouse testes after 60Co gamma-Radiation. Iran Biomed J. 2008;12:35-42.
10. Absalan F, Movahedin M, Mowla SJ. Evaluation of apoptotic genes expression and its protein after treatment of cryptorchid mice. Iran Biomed J. 2012;16:77-83.

11. Ma W, An L, Wu Z, et al. Efficient and safe recipient preparation for transplantation of mouse spermatogonial stem cells: pretreating testes with heat shock. Biol Reprod. 2011;85:670-7.
12. Mclean DJ, Russell LD, Griswold MD. Biological activity and enrichment of spermatogonial stem cells in vitamin A-deficient and hyperthermia-exposed testes from mice based on colonization following germ cell transplantation. Biol Reprod. 2002;66:1374-9.
13. Clouthier DE, Avarbock MR, Maika SD, Hammer RE, Brinster Rl. Rat spermatogenesis in mouse testis. Nature. 1996;381:418-21.
14. Cox Am, Patel H, Gelister J. Testicular torsion. Br J Hosp Med (Lond). 2012;73:C34-6
15. Lysiak JJ, Turner SD, Turner TT. Molecular pathway of germ cell apoptosis following ischemia/reperfusion of the rat testis. Biol Reprod. 2000;63:1465-72.
16. Lysiak JJ, Turner SD, Nguyen QA, Singbartl K, Ley K, Turner TT. Essential role of neutrophils in germ cell-specific apoptosis following ischemia/reperfusion injury of the mouse testis. Biol Reprod. 2001;65:718-25.
17. Johnsen SG. Testicular biopsy score count–a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. Hormones. 1970;1:2-25.
18. Van Pelt AM, Morena AR, Van Dissel-Emiliani FM, et al. Isolation of the synchronized A spermatogonia from adult vitamin A-deficient rat testes. Biol Reprod. 1996;55:439-44.
19. Izadyar F, Spierenberg GT, Creemers LB, Den Ouden K, De Rooij DG. Isolation and purification of type A spermatogonia from the bovine testis. Reproduction. 2002;124:85-94.
20. Dirami G, Ravindranath N, Pursel V, Dym M. Effects of stem cell factor and granulocyte macrophage-colony stimulating factor on survival of porcine type A spermatogonia cultured in KSOM. Biol Reprod. 1999;61:225-30.
21. Ogawa T, Arechaga JM, Avarbock MR, Brinster Rl. Transplantation of testis germinal cells into mouse seminiferous tubules. Int J Dev Biol. 1997;41:111-22.
22. Brinster CJ, Ryu BY, Avarbock MR, Karagenc L, Brinster RL, Orwig KE. Restoration of fertility by germ cell transplantation requires effective recipient preparation. Biol Reprod. 2003;69:412-20.
23. Ogawa T, Ohmura M, Yumura Y, Sawada H, Kubota Y. Expansion of murine spermatogonial stem cells through serial transplantation. Biol Reprod. 2003;68:316-22.
24. Zhang Z, Renfree MB, Short RV. Successful intra-and interspecific male germ cell transplantation in the rat. Biol Reprod. 2003;68:961-7.
25. Hermann BP, Sukhwani M, Lin CC, et al. Characterization, cryopreservation, and ablation of spermatogonial stem cells in adult rhesus macaques. Stem Cells. 2007;25:2330-8.
26. Honaramooz A, Behboodi E, Hausler CI, et al. Depletion of endogenous germ cells in male pigs and goats in preparation for germ cell transplantation. J Androl. 2005;26:698-705.
27. Ryu By, Orwig KE, Oatley JM, et al. Efficient generation of transgenic rats through the male germline using lentiviral transduction and transplantation of spermatogonial stem cells. J Androl. 2007;28:353-60.
28. Hamra Fk, Gatlin J, Chapman KM, et al. Production of transgenic rats by lentiviral transduction of male germ-line stem cells. Proc Natl Acad Sci U S A. 2002;99:14931-6.
29. Ogawa T, Dobrinski I, Brinster R. Recipient preparation is critical for spermatogonial transplantation in the rat. Tissue Cell. 1999;31:461-72.
30. Turner T. Acute experimental testicular torsion. No effect on the contralateral testis. J Androl. 1985;6:65-72.
31. Turner T, Brown K. Spermatic cord torsion: loss of spermatogenesis despite return of blood flow. Biol Reprod. 1993;49:401-7.
32. Cosentino M, Nishida M, Rabinowitz R, Cockett A. Histopathology of prepubertal rat testes subjected to various durations of spermatic cord torsion. J Androl. 1986;7:23-31.
33. McCord JM. Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med. 1985;312:159-63.
34. Bergh A, Damber J, Marklund SL. Morphologic changes induced by short-term ischemia in the rat testis are not affected by treatment with superoxide dismutase and catalase. J Androl. 1988;9:15-20.
35. Azizollahi S, Babaei H, Derakhshanfar A, Oloumi M. Effects of co-administration of dopamine and vitamin C on ischaemia-reperfusion injury after experimental testicular torsion-detorsion in rats. Andrologia. 2011;43:100-5.
36. Agarwal A, Said TM. Oxidative stress, DNA damage and apoptosis in male infertility: a clinical approach. BJU Int. 2005;95:503-7.
37. Honaramooz A, Megee SO, Dobrinski I. Germ cell transplantation in pigs. Biol Reprod. 2002;66:21-8.
38. Turner T. On unilateral testicular and epididymal torsion: no effect on the contralateral testis. J Urol. 1987;138:1285-90.
39. Russell L, Ettlin R, Sinha Hikim A, Clegg E. Mammalian spermatogenesis. Histological and histopathological evaluation of the testis. Clearwater, FL; Cache River Press; 1990. p. 1-40.
40. Hernández-Franyutti A, Uribe MC. Seasonal spermatogenic cycle and morphology of germ cells in the viviparous lizard Mabuya brachypoda (Squamata, Scincidae). J Morphol. 2012;273:1199-213.
41. Kubota H, Brinster RL. Technology insight: in vitro culture of spermatogonial stem cells and their potential therapeutic uses. Nat Clin Pract Endocrinol Metab. 2006;2:99-108.
42. Brinster RL. Male germline stem cells: from mice to men. Science. 2007;316:404-5.
43. Oatley JM, Reeves JJ, Mclean DJ. Biological activity of cryopreserved bovine spermatogonial stem cells during in vitro culture. Biol Reprod. 2004;71:942-7.