Graft Efficiency of Co-Cultured Spermatogonial Cells Using Sperm Assay in Epididymal Lumen of Recipient Mice

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Abstract

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Introduction: Transplantation of germ cells restores the male fertility. Nevertheless, a lot of questions remain incompletely resolved. The aim of this study was to evaluate in vitro colonization efficiency of germ cells and sperm production capacity of spermatogonial cells before and after culture by sperm number assay in epididymis of recipient mice.

Material and Methods: We developed a Sertoli cell feeder in a co-culture system with spermatogonial cells and the cells were co-cultured for 2 months. The cells were isolated from mouse neonates. Colony assay was performed during culture using light microscopy. The transplanted cells were traced using BrdU incorporation. Sperm parameters were assessed 2 months after transplantation.

Results: Our findings showed that spermatogonial cells created colonies during culture. Transplantation of fresh spermatogonial cells at a concentration of 2×10^5 cells/ml did not show significant differences. However, after transplantation of 2×10^5 cells/ml cultured for 2 weeks, the number of epididymal sperms in recipients increased significantly in groups with more fresh cells.

Conclusion: Epididymal sperm number in recipient mice can be increased by enrichment of type A spermatogonial cells using an in vitro co-culture system. Other important factors include the source of donor cells and the number of transplanted cells.

Keywords: Graft, Spermatogonial cells, Spermatogenesis, Sperm assay

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Introduction

Spermatogenesis is a complex developmental process consisting of sequential, highly organized steps of cell proliferation and differentiation resulting in generation of functional spermatozoa (1).

Many growth factors, hormones, and interactions of germ cells with Sertoli cells regulate these processes, and the failure of each process can lead to male infertility (2). Germ cell transplantation procedures combined with developments in culturing or enriching germ cell populations have applications of paramount importance in medicine, basic sciences, and animal reproduction. In addition, these techniques can serve as an alternative approach for gonadal protection and fertility preservation in patients with cancer (3). *In vitro* culture of germ cells is useful for enrichment of spermatogonial cells for further studies on genetic modification, gene transfection, *in vitro* differentiation, cryopreservation, restoration of male fertility by spermatogonial cell transplantation, etc. So, the colonization efficiency of spermatogonial cells after *in vitro* co-culturing with Sertoli cells was investigated in this study. In initial mouse studies, co-culture with an STO feeder cell line was demonstrated to support the survival of spermatogonial stem cells for several months (4). Over the past 10 years, all definitive reports of maintenance of spermatogonial stem cells *in vitro* have relied on co-culture with a feeder cell monolayer (4, 5). Sertoli cells have vital roles in the spermatogenic function of the testis for many reasons (6). These somatic cells generate and maintain the cyto-architecture of the germinal epithelium, produce nutrients that provide energy substrates to the germ cells and, in the primate, represent the only cellular component of the blood-testis barrier (7). Receptor tyrosine kinase c-kit is expressed in germ cells;

and its ligand, stem cell factor (SCF), is expressed in the Sertoli cells of the testis. The interaction between c-kit and SCF is essential for the maintenance and/or mitosis of differentiating type A spermatogonia (8).
Transplantation of spermatogonial stem cells from a donor mouse testis into the seminiferous tubules of a recipient mouse testis has been shown to result in donor-derived spermatogenesis. However, a complete spermatogenesis *in vitro* has not yet been obtained in any species (9) and new approaches based on male germ cell transplantation and testicular tissue grafting can be applied to generate only a limited number of sperm cells (10). Therefore, enhancement of transplantation efficiency has important clinical implications. Spermatogonial stem cell transplantation has been demonstrated in rodents (11, 12) and goats (13), and bovine spermatogonial stem cells have been indicated to be capable of colonizing recipient mouse seminiferous tubules, but not differentiating into spermatozoa (14, 15). The development of a spermatogonial transplantation technique has provided a new treatment strategy for male infertility (11). Since spermatogonial stem cells self-renew and differentiate into proliferating spermatogonia, they provide a unlimited supply of mature spermatozoa (16, 17). Therefore, establishment of better conditions for increasing epididymal sperm parameters developed from spermatogonial cells after transplantation for restoration of male fertility has important clinical implications. The use of *in vitro* culture techniques and transplantation have opened a new avenue to study of the biology of spermatogonial stem cells hoping for better understanding of male germ cell biology and regulatory factors of male fertility . Despite invaluable previous findings, the *in vitro* colonization efficiency of type A spermatogonial cells and *in vivo* sperm production capacity of spermatogonial stem cells before and after culture following transplantation into epididymal lumen of the recipient mouse (in infertility models of busulfan-treated mice) have not yet been well characterized and could become an important strategy for preserving male gonadal function and assessment of transplanted testis.

Material and Methods

Preparation of donor mice

Animals

Male neonatal (6 days old; n = 120) NMRI mice, derived from original stocks obtained from Razi Laboratory (Tehran, Iran), were maintained with free access to food and water at the animal facilities of Tarbiat Modarres University. The research was conducted in accordance with the National Research Council guidelines.

Cell isolation and culture

Bilateral testes from 10 to 20 neonatal 6 days old NMRI mice were collected for cell suspension. They were placed on ice and transferred to the laboratory within 30 minutes. After decapsulation, the testes were minced into small pieces and suspended in Dulbecco's modified Eagle medium (DMEM; Gibco Life Technology), supplemented with 14 mM $NaHCO_3$ (Sigma, St. Louis, MO, USA), single-strength non-essential amino acids, 100 IU/mL penicillin, 100 µg/mL streptomycin, and 40 µg/mL gentamycin (all from Gibco). The minced pieces of testes were suspended in DMEM, containing 0.5 mg/mL collagenase/dispase, 0.5 mg/mL trypsin, and 0.08 mg/mL DNase for 60 minutes under shaking at 37°C. All enzymes were purchased from Sigma Chemical Co. (St. Louis, MO, USA). After three washes in the DMEM and removal of most of the interstitial cells, a second digestion (45 minutes at 32°C) was performed in DMEM by adding fresh enzymes to the seminiferous cord fragments. Most of the cell aggregates remaining after this treatment were sheared gently by repeated pipetting with a Pasteur pipette for 5 minutes. The cells were separated from the remaining tubule fragments by centrifugation at 400 RPM for 2 minutes. After filtration through a 70-µm nylon filter, the medium was aspirated, the cells were washed twice, and fresh medium was added.

Preparation of lectin-coated dishes

Coated plastic dishes were prepared by incubation with a solution of 5 µg/ml of Datura Stramonium Agglutinin (DSA; Sigma) in phosphate-buffered saline (PBS) at 37°C for 60 minutes, followed by extensive washing with PBS, supplemented with 0.5% bovine serum albumin (BSA; Sigma).

Sertoli cell isolation

The Sertoli cells were isolated using a procedure described by Scarpino *et al.* (18) with some modifications. The mixed population of the cells obtained by enzymatic digestion was placed on lectin-coated dishes at a concentration of 1.5×10^5 cells/cm^2 and incubated for 1 hour at 32°C in a humidified atmosphere of 5% CO_2. After the incubation, the non-adhering cells were collected by washing twice with medium. Alternatively, 48 hours after having been plated on the lectin-

coated dishes, the Sertoli cells were detached by EDTA-trypsin treatment (0.02% EDTA, 01% trypsin in Ca^{2+}, and Mg^{2+}-free PBS) for 5 minutes at 37°C, counted and adjusted to desired densities into each well of a multidish 24-well plate (1.5×10^5 cell/cm^2) for secondary culture in DMEM at 32°C in the presence of 10% fetal bovine serum (FBS; Gibco). This method helped isolate the Sertoli cells with more than 95% purity.

Spermatogonial cell isolation

After isolation of the Sertoli cells using DSA lectin, the spermatogonia that remained in suspension were collected and kept at 32°C in a humidified atmosphere in the presence of 10% FBS. More than 95% of the cells were spermatogonial cells.

Cell number and viability

The number of the Sertoli and spermatogonial cells was determined with a hemacytometer. Cell viability was evaluated by means of the dye exclusion test (0.04% trypan blue solution).

Co-culture of Sertoli and spermatogonial cells

Two days after above procedure, the Sertoli cells formed a confluent layer, and spermatogonial cells (23×10^3 cell/ml) were co-cultured on top of them. The cells were then grown in DMEM at 32°C in the presence of 10% FCS for two months.

Colony assay

The assay of spermatogonial cell-derived colonies was commenced after 5 days of co-culture and carried out every 3 days after the appearance of colonies during culture. A Leica inverted-phase microscope was used to determine the colony numbers, with their diameters being measured by ocular lenses. Results from three replications were used to calculate the mean and SDM.

Identity confirmation of Sertoli and spermatogonial cells

Morphological and histochemical evaluation

The relative size estimate of the spermatogonial cells and Sertoli cells was determined using light microscopy with appropriate ocular lenses. For alkaline phosphatase (Sigma) assay, slides were fixed in a solution containing 2.5 ml citrate, 6.5 ml acetone, and 0.6 ml formaldehyde in distilled water for 10 minutes and incubated for 30 minutes in a solution containing 0.5 mg/ml Fast Red Violet and 40 µl/ml α-naphtol phosphate (0.25% solution). After having been rinsed in water, the samples were mounted and observed under light microscopy.

Immunohistochemistry

For cytokeratin immunohistochemistry, the cells were fixed for 30 minutes in 4% paraformaldehyde at room temperature before being rinsed with PBS. Following permeabilization by 0.2% triton X-100 to facilitate antibody penetration, the cells were washed with PBS, supplemented with 0.2% BSA. Extraneous antibodies were blocked with 10% goat serum. The slides were then incubated for 1 hour at 37°C with a mouse monoclonal anti-cytokeratin pan-antibody (diluted 1:100; Boheringer-Mannheim, Germany) described as a marker for Sertoli cells (19). After extensive washing with PBS, the second antibody (goat FITC-conjugated anti-mouse IgG; diluted 1:100) was applied for 45 minutes. The control slides were under similar conditions, except for the removal of the first antibody.

For c-kit (CD117) immunohistochemistry of the obtained clusters, the colonies grown on the glass slides were fixed in 1% formalin in PBS for 10 minutes. The slides were subsequently washed in PBS, treated with a blocking solution containing 10% normal goat serum (Vector, Burlingame, CA) for 20 minutes, and rinsed again in PBS. After the blocking step, the slides were incubated for 60 minutes with a mouse anti-c-kit (CD117; Santa Cruz Biotechnology, USA) polyclonal antibody (1:100) diluted in PBS. Having been rinsed three times in PBS, the slides were incubated for 1 hour with goat anti-mouse secondary antibody conjugated to FITC (1:100; Gibco) diluted in PBS. All the incubations were performed in a moist chamber at 37°C with 1.5-3% normal blocking serum. The slides were then mounted with 90% glycerol in PBS. Examination was carried out using a fluorescence microscope with appropriate filters.

Preparation of recipient testis

Adult NMRI mice of 6 to 8 weeks old were purchased from Razi institute (Karaj, Iran). Fresh busulfan powder, a white or almost white crystalline powder (Sigma B2635, Sigma, Poole, UK) was dissolved in *dimethylacetamide (DMA)* 33% (wt/wt) and *polyethylene glycol 400,* 67% (wt/wt) at room temperature. Mice were weighted and then given a single intraperitoneal injection of busulfan at a dose of 20 mg/kg. In each experiment, at least six animals were received busulfan. All animal experimental protocols were approved by the Institutional Animal Care

and Use Committee of Tarbiat Modarres University.

Transplantation

BrdU incorporation

For tracing the transplanted cells, the co-cultured cells were incorporated with BrdU (Sigma) by adding 0.1mM BrdU of the culture medium (final concentration) 72 hours before transplantation. The co-cultured cells were detached using EDTA-trypsin treatment (0.02% EDTA-01% trypsin in Ca^{2+}-and Mg-free PBS) for 5 minutes at 37°C, and the Sertoli cells were isolated by DSA lectin immobilization. As a result, the spermatogonia that remained in suspension were collected and transplanted. Transplantation was carried out 2 weeks after culturing.

Immunocytochemistry of BrdU incorporated cells after transplantation

For immunohistochemical staining, the sections were first deparaffinized and rehydrated, and then they were boiled in the oven (60ºC) for 120 minutes and cooled in PBS. The sections were activated again by adding 1% hydrogenperoxide at room temperature for 20 minutes and placed into 2N HCL at 60ºC for 30 minutes. Subsequently, they were put into 0.1 M borate buffer (pH 8.5) at room temperature for 20 minutes before being washed by 0.01 M PBS. After having been blocked in 10% normal goat serum (Sigma) at 37ºC for 30 minutes, the sections were treated with monoclonal antibody against BrdU diluted at 1:500 and left for 48 hours at 4ºC. After extensive washing with PBS, the second antibody (goat peroxidase-conjugated anti-mouse IgG; diluted 1:100) was applied for 2 hours at room temperature. The sections were rinsed again in PBS. After the sections were extensively washed in PBS, they were incubated with DAB solution (Sigma, 5 mg powder in 5 ml PBS and 0.33 µl/ml H_2O_2 35%) for 30 minutes. The sections were washed in MilliQ water for 20 minutes. Finally, they were dehydrated and mounted with entellane. Control staining consisted of the same process excluding reaction against BrdU.

Recipient Mice and Transplantation Procedure

The spermatogonial cells with a purity of more than 95% were transplanted into the seminiferous tubules of the recipient mice, treated with busulfan (20 mg/kg i.p.) at the age of 6 to 8 weeks. The busulfan-treated recipients were devoid of endogenous spermatogenesis at the time of transplantation (~6 weeks after busulfan treatment) (12). The adult recipient mice were anesthetized by Ketamine 10% and Xylazine 2% (Alfasan, Woerden, Netherlands).

Approximately 2×0^5 to 4×0^6 donor spermatogonial cells in 20 µl DMEM were injected into the seminiferous tubules of one testis of each recipient mouse; the contralateral testis was served as an internal control.

Analysis of Recipient Testes

The transplanted and control testes of the recipient mice were examined 8 weeks after transplantation. The testes were fixed in 4% paraformaldehyde, dehydrated, and embedded in paraffin. The sections were then immunostained with a primary anti-BrdU (Sigma) so that the donor-derived spermatogenesis could be visualized.

Sperm parameter assessment

The epididymis was placed in 1000 µl phosphate buffer saline (PBS) at Ph 7.4 and minced into small pieces, and then incubated at 37° for 30 min. Sperm parameters were monitored by light microscopy. Viability of sperms was assessed by determining the proportion of sperms excluding vital dye (25% eosin solution). 7 µl of eosin solution was add to 20 µl of cell suspension after incubation and mixed thoroughly, and eosin stains the head of dead sperms, but dose not permeate the membrane of living cells, leaving them unstained. Motility of sperms was assessed by determining the proportion of motile sperms. Finally, sperm number was also determined with a hemacytometer count.

Statistical Analysis

Results are expressed as mean ± standard deviation (S.D.). The statistical significance of any difference between mean values was determined using Student's *t-test*; $p<0.05$ was considered as significant.

Results

In vitro colonization efficiency of the spermatogonial cells

Isolated fresh type A spermatogonial cells were co-cultured on feeder layer of the Sertoli cells. Our results indicated that spermatogonial derived colonies were round or oval shape with different diameters. During culture, the number of spermatogonial derived colonies and their diameters increased until the 14^{th} day ($p<0.05$). The cumulative lateral spread of colonies was at approximately 22.7 µm per day and the size of the two-month colonies was 388.3 µm. However, the number of

colonies decreased after 2 months of culture (Table 1). The number of cells per colony depended on the size of colony; about 3400 cells were counted using hemacytometer in the colonies with diameter of nearly 196 (µm).

Transplantation efficiency of fresh and cultured spermatogonial cells

In this study, the role of culturing in the transplantation efficiency was investigated using sperm number assessment in epididymal lumen of the recipient's testes. BrdU was added 72 hours before transplantation, and staining was examined just before transplantation. More than 85% of the total transplanted cells were labeled with BrdU before transplantation. Two months after transplantation of the cells into seminiferous tubules of left testes, transplanted spermatogenic cells, as detected by tracing BrdU incorporated cells, were found in the tubule cross-sections of left recipient mouse testes (Fig 1).

Transplanted spermatogonial cells restored spermatogenesis in the recipient testes. No BrdU positive cells were found in the non-transplanted group (right testis which considered as sham group), confirming the specificity of this identification method.

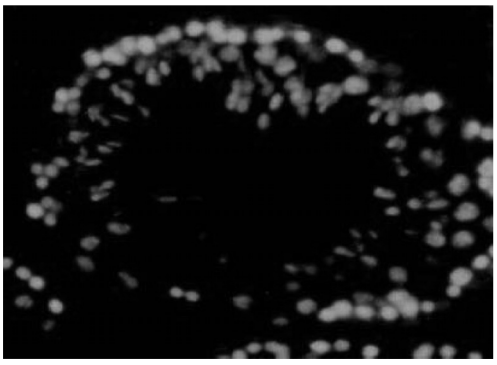

Fig 1: Tracing of transplanted cells using BrdU incorporation, showing that spermatogonial cells restored spermatogenesis in seminiferous tubule of recipient mouse after transplantation. Magnification: ×200.

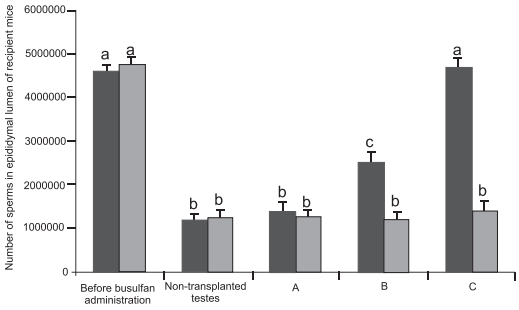

Fig 2: The number of sperms in right (sham) and left epididymal lumens of recipient mice after transplantation with three different cell concentrations (A, 2×10^5; B, 2×10^6; C, 4×10^6cells/ml) into seminiferous tubules of left testes. Non-transplanted testes considered as control group. Data represents means ±SEM of 6 independent experiments. Bars with different superscripts show significant differences ($p<0.05$).
■ The number of sperms per ml in left epididymal lumen.
■ The number of sperms per ml in right epididymal lumen.

The proportion of donor-derived spermatozoa in epididymal lumen of recipients showed a significant correlation with the number of transplanted cells (Fig 2).

Colonization efficiency was assessed using the number of sperms in left epididymal lumen of recipient mice. After transplantation of 2×10^5 cells/ml into the seminiferous tubules of left testes, epididymal sperm parameters in the transplanted group with fresh cells were not significantly different from non-transplanted groups (the control and sham groups).

When 2×10^6 cells/ml (fresh cells) were transplanted, the number of epididymal sperms increased compared to the non-transplanted groups ($p<0.05$). Transplantation of more fresh cells (4×10^6) increased epididymal sperm numbers significantly compared to the group which received 2×10^6 fresh cells/ml (Fig 2).

After transplantation of 2×10^5 cells/ml which co-cultured for 2 weeks, the number of epididymal sperms increased significantly ($p<0.05$) compared to the non-transplanted groups (Fig 3).

Table 1: The mean colony number and diameter (***µm***) during a 2 month culture ± SD

	Mean colony number and diameter in different cultures ± SD				
Parameter	5th day	8th day	11th day	14th day	60th day
Colony number	0*	18±3.6*	32±7.77*	40±9.19*	31±6.8
Colony diameter	0*	196±38.16*	244.8±42.56*	332.43±66.4 *	388.3±69*

The experiments were repeated 6 times in all groups.
* Significant difference in the same row ($p<0.05$).

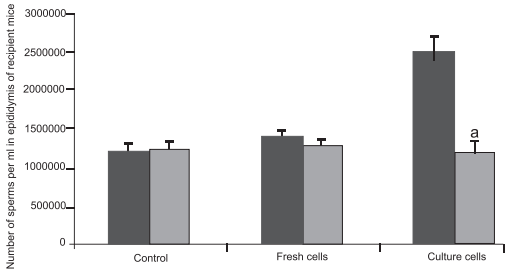

Fig 3: The number of sperms in right (sham) and left epididymal lumens of recipients after transplantation of 2×10^5 cells/ml into seminiferous tubules of left testes in the experimental groups. Data represents means ±SEM of 6 independent experiments. Bars with different superscripts show significant differences ($p<0.05$).
■ The number of sperms per ml in left epididymal lumen.
■ The number of sperms per ml in right epididymal lumen.

Assessment of other sperm parameters (e.g. viability and motility) did not show any significant change (data not shown).

Discussion

New approaches based on male germ cell transplantation can be used to generate sperm and could therefore be considered important new avenues for restoration of fertility in oncological patients (10, 20). However, a lot of questions remain incompletely resolved in animal models and of course in humans, including stem cell enrichment and transplantation procedure. While a complete spermatogenesis *in vitro* has not yet been obtained in any species (9), *in vitro* culture may increase the rate of successful transplantation (21).

Spermatogonial transplantation is the only method that restores natural fertility to the host, and it will be important to identify circumstances in which this technique may be applied in clinical situations (22). So, enhancement of transplantation efficiency is an important matter. Thus, to use any strategy for enhancement of transplantation efficiency, it is necessary to assess sperm production capacity of transplanted cells. So, a new strategy to assess the transplantation is useful, especially a clinically applicable approach. Assessment of the presence and proportion of donor-derived sperm production will require a permanent or long-term traceable labeling system for donor cells. Fluorescent markers provide a rather short-term labeling tool (23). Currently, two ways are used for assessment of transplantation: by colonization assessment in the testis after transplantation and by mating transplanted mouse. The former is not applicable in clinical settings, because the extraction of the testis and then colonization assessment is not reasonable. Furthermore, the colony number dose not necessarily represents the number of stem cells transplanted. It is possible that a single colony derived from several stem cells or two or more colonies merged during their development (24). In the latter one, assessment of transplantation follows the *all or none* rule, which means transplanted mouse is either able or unable to create offspring after mating. On other hand, assessment of the sperm parameters in the epididymal lumen is of particular importance when the transplanted animal could not create offspring. This study proposed a new approach, in which transplantation was assessed by sperm count in epididymis using a simple aspiration of sperms from epididymal lumen, which is both simple and practical in clinical settings. Thus, based on previous studies, we believe that the success of spermatogonial transplantation must be evaluated by sperm parameter assay in the recipient epididymal lumen. So, we infused spermatogonial cells into azoospermic mouse testes and showed that restoration of spermatogenesis in the testis following spermatogonial transplantation influences epididymal sperm parameters in the recipient mouse.

Spermatogonial stem cells represent an attractive cell population for preservation of male germ lines. These cells, due to their self-renewal properties, have an enormous capacity to regenerate from a small basal population and to produce unlimited numbers of mature spermatozoa (25, 26).

The approaches based on male germ cell transplantation and testicular tissue grafting can be used to generate only a limited number of sperm cells (10). On the other hand, the number of spermatogonial stem cells in the adult mouse testis is low, i.e. 0.03% of all testicular germ cells (27). As only a limited number of stem cells can be recovered from a small biopsy sample from a patient (28), spermatogonial stem cell enrichment is an important strategy for enhancement of transplantation efficiency, especially in clinical settings. Therefore, it is necessary to consider *in vitro* culture of spermatogonial cells, which may increase the rate of transplantation efficiency (21). Thus, this investigation was performed to test whether culturing and increasing the number of transplanted cells could improve epididymal sperm number in the recipient. The donor neonatal mice used in this study were 6 days old, a developmental stage in which Sertoli (84%) and type A spermatogonia (16%) are the only cell types

present within the seminiferous tubule (29, 30). The size, morphology, and alkaline phosphatase activity of the Sertoli and type A spermatogonial cells were utilized as parameters for characterization (18, 31, 32). In addition, detection of specific markers was carried out using cytokeratin and c-kit immunocytochemistry (19, 33). It has been reported that even though Leydig cells express c-kit, their precursors differentiate from mesenchymal-like stem cells on day 21 postnatal (34). So, we assume that the colonies may have been largely derived from the spermatogonial cells. Further evidence for confirmation of the spermatogenic nature of these colonies comes from their ability to create spermatogenesis in the recipient testes after transplantation. Because spermatogonial stem cells which are a subclass of type A spermatogonial cells have no known unique biochemical markers or characteristics, the presence of them in a population of cells can be evaluated by transplanting into an azoospermic recipient (35, 36).

Our *in vitro* culture system for spermatogonial-cell colonization can provide conditions for *in vitro* enrichment of spermatogonial cells for such further studies as cryopreservation, restoration of male fertility by transplantation of spermatogonial cells, genetic modification, gene transfection, *in vitro* differentiation, etc.

While in present study about 4600 spermatogonial stem cells/ml resulted in 8 colonies after 2 wk culture, *in vivo* studies demonstrated that 8 colonies resulted 7 weeks after transplantation of 158 spermatogonial stem cells/ml (24). Thus, it could be assumed that while *in vitro* colonization is faster than *in vivo* one, more colonies formed *in vivo*. Furthermore, *in vitro* cumulative lateral spread of the colonies were shown (6 and 22.7 µm per day in the frozen/thawed and control groups, respectively) and the size of a 2-month colony was 385 µm. However, the cumulative lateral spread of spermatogenesis in a transplanted tubule is rapid and moving at approximately 55-60 µm per day (37); the size of a 2-month colony is 2 mm (38).

The number of sperms in the epididymis of recipient mice after transplantation of 2×10^5 fresh cells was significantly lower than spermatogonial cells co-cultured with Sertoli cells at 2 weeks. Sertoli cell growth factor which is secreted by Sertoli feeder cells may have contributed to the enhanced stem cell maintenance in 2-week cultures (39). Obviously, the number of stem cells in the donor cell population is a critical factor to increase colonization (17, 40). However, different concentrations of the cell suspension have been used for transplantation in the previous studies, from 3.3×10^5 up to 20×10^6 cells/ml (24, 41). So, transplantation of more fresh cells (2×10^6 cells/ml) in this study showed that more concentrated stem cell suspensions result in more epididymal sperm numbers.

In the present study, an average of 52% to 70% of the all sperms in epididymal lumen of recipients were donor-derived after 2 months (in transplanted group with 2×10^5 cultured cells/ml and 4×10^6 fresh cells/ml, respectively), while the colonization efficiency of transplanted stem cells has been reported to be 5 to 10% three months after transplantation (12, 38, 40, 41). In another study, Izadyar *et al.* (42) were the first researchers who demonstrated the successful autologous transplantation of bovine spermatogonial stem cells with a yield of about 15% donor-derived spermatozoa 2.5 months after transplantation. Sperm assay in the epididymal lumen after transplantation of different cell suspensions (2×10^5 and 4×10^6 cells/ml) showed an almost linear correlation between the number of injected cells and the number of epididymal sperms in recipient mice. These findings were consistent with a report from Dobrinski *et al.* (41) who demonstrated a similar correlation between the number of injected cells and the degree of colonization in the recipient testes. Thus, the present results demonstrated the first successful application of epididymal sperm number in recipient for assessment of transplantation and highlight the promising aspects of using co-culture system prior to transplantation in humans.

Conclusion

Important factors in the transplantation efficiency include optimization of transplantation procedure using an *in vitro* culture system for colonization of type A spermatogonial cells, simple and efficient approach to graft assessment, the source of donor cells, and finally the number of transplanted cells.

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ارزیابی سطح تولید اسپرم در اپی دیدیم موش گیرنده بعد از پیوند سلول‌های اسپرماتوگونی غنی شده از طریق هم کشتی

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چکیده

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*** هدف:** غنی سازی سلول‌های بنیادی اسپرماتوگونی در محیط آزمایشگاه و افزایش میزان تولید اسپرم در موش گیرنده از طریق هم‌کشتی سلول‌های اسپرماتوگونی قبل از پیوند

*** مواد و روش‌ها:** برای رسیدن به اهداف این پژوهش سلول‌های اسپرماتوگونی روی لایه‌ای ازسلول‌های سرتولی که از بیضه موش نوزاد جدا شده بودند برای مدت ۲ ماه کشت شدند. در طی کشت ارزیابی کلونیزاسیون به کمک میکروسکوپ نوری انجام شد. سلول‌های پیوند شده به کمک BrdU ردیابی و پارامترهای اسپرم ۲ ماه بعد از پیوند بررسی شدند.

*** یافته‌ها:** نتایج پژوهش حاضر نشان می‌دهد که سلول‌های بنیادی اسپرماتوگونی در طی کشت ،کلونی ایجاد کرده و پیوند سلول‌های غنی‌سازی شده از طریق کشت و افزایش غلظت سلول‌ها در سوسپانسیون پیوندی میزان تولید اسپرم را در اپی‌دیدیم موش گیرنده افزایش می‌دهد.

*** نتیجه‌گیری:** غنی‌سازی سلول‌های اسپرماتوگونی به کمک سیستم هم‌کشتی، منبع سلول‌های دهنده پیوند و نهایتا تعداد سلول‌های پیوند شده، راه‌های اساسی افزایش تعداد اسپرم در موش گیرنده می‌باشد.

کلیدواژگان: پیوند، سلول‌های اسپرماتوگونی، اسپرماتوژنز، ارزیابی اسپرم