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Current Approach to Spermatogonial Stem Cells in Vitro Maturation

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ABSTRACT

The studies conducted to determine the stage of its usability in prospective fertility restoration of testicular tissues taken from prepubertal patients undergoing oncology treatment were screened. In addition, the current status of spermatogonial stem cell cultures, testicular tissue cultures, and testicular organoid research and their potential in fertility restoration were examined.

Spermatogonial stem cells are only found in prepubertal testicular tissue. Germinal serial cells are not found. Since spermatozoa are not produced in this period, spermatogonial stem cells are stored by freezing in the form of testicular tissue pieces or testicular cell suspension. It is not yet clear how to ensure the maturation of freeze-thawed or fresh spermatogonial stem cells for fertility reconstruction when it is necessary. The spermatogonial stem cells can be placed in their original niche by maintaining the vitality of the seminiferous tubules in vitro. Then, it can be transplanted to the recipient.

Many hypotheses suggested that that maturation can be achieved via such as two-dimensional, three-dimensional tissue cultures. To ensure differentiation and proliferation of spermatogonial stem cells in three-dimensional cultures, it is necessary to ensure the long-term viability of the seminiferous tubules in vitro or solve creating an environment similar to the seminiferous tubules niche.

In this review article, spermatozoa could be obtained in three-dimensional culture. However, the application of this system in different laboratories and the provision of the setup involves various difficulties.

Standard organoids and organoid scaffolds that can be developed for three-dimensional cultures seem to be more preferable.

Keywords: In vitro maturation, three-dimensional culture, testicular organoid, seminiferous tubules niche.

1. INTRODUCTION

The spermatogonial stem cells (SCC) of patients who will undergo oncology treatment in the prepubertal period can be stored by slow freezing and vitrification. Currently, 1033 testicular tissues belonging to boys between the ages of 3 months and 18 years are stored in the cryobank for future use in centers in Europe and North America (1).

Reaching 80% of survival with the treatment methods developed in childhood cancer diseases necessitates the development in the use of prospective SCC in fertility restoration (2). Can mature spermatozoa be obtained from SCC in vitro? Can spermatogenesis be achieved? Can meiosis occur in immature testicular tissue? It requires answering these questions. In the future, it is not clear how the SCC can be used for fertility restoration and how their maturation can be achieved. The testicular tissue of oncology patients in the prepubertal period contains not only SCC but cancer cells. Therefore, some problems need to be overcome, such as SCC isolation from cancer cells, cryopreservation, transplantation, and maturation of these cells. Here, we examined current developments in the stage of in vitro maturation applications with two-dimensional (2D) and three-dimensional (3D) cultures applied to testicular tissues used in fertility restoration with a literature review.

Some researchers have sought solutions that have been sought by experimental autologous, homologous, or xenotransplantation applications of fresh or frozen-thawed tissues (3, 4, 5).

In order to ensure full spermatogenesis in vitro, providing microenvironment conditions suitable for SCC is seen as

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the target solution. The real microenvironment should be provided in culture, and in vivo maturation (differentiation and proliferation) of the SCC should be realized. Therefore, in fertility restoration, some researchers have tried to ensure in vitro maturation of the SCCs by keeping the seminiferous tubule tissue alive in vitro and preserving its viability (6). Furthermore, in vitro culture studies of fresh or freeze-thawed SCCs are ongoing, and 2D and 3D cultures, organoids, organoid scaffolds are tried to be developed. They try to provide in vitro maturation of SCCs on the organoid scaffold, which can best imitate morphological and physiological in vivo conditions (7, 8). Developments in this subject will also shed light on the treatment of adults with maturation arrest. Advances will significantly contribute to the treatment of fertility restoration for prepubertal oncology patients and adults with maturation arrest.

1.1. Niche of Spermatogonial Stem Cells In Seminiferous Tubules

For in vitro maturation to occur, it is necessary to know the change and development of the SCCs and the morphological and physiological conditions of the niche where these cells are located. Male reproductive cells undergo a very complex process until they pass through the embryonal, fetal, postnatal, and pubertal periods and become mature spermatozoa. Stem cells migrate from the seminiferous tubules lumen to the base in the gonocyte stage and settle on the basement membrane. Reproductive cells located on the basement membrane form spermatogonium (9, 10).

Spermatogoniums form SCCs that can renew and differentiate themselves through mitosis (11). Sertoli cells are somatic host cells lined up on the basement membrane in the seminiferous tubules.

While the gonads develop between 4-8 weeks in the organogenesis phase during the embryonal period, the surface begins to form within the gonadal cords made by the mesodermal epithelium. Sertoli cells act as a bridge between the vascular area and the germ cells. SCCs are lined up along the lateral surfaces of Sertoli cells from the basal to the lumen. Therefore, it actively manages spermatogenesis and provides nutrition. SCCs have settled in a special micro-environment within the seminiferous tubules, whose control and testis balance. The survival, proliferation, and differentiation of SCCs take place in this microenvironment (12). The SCC niche can be defined as a micro-environment that fixes the SCCs to preserve their origins. Tissue architecture is essential for the differentiation and proliferation of SCCs. Niche is a microenvironment that includes morphology and physiological function together.

It is a local tissue microenvironment that directly protects and regulates all cells of the germinal series (13). Somatic Sertoli and Leydig cells and peritubular myoid cells direct this niche, located in the basal compartment required for spermatogenesis. Sertoli and peritubular myoid cells provide the adhesion molecules that allow SCCs to bind to the basement membrane (14). Although Leydig cells and peritubular myoid cells do not directly contact SCC, they control the SCC niche through Sertoli cells (15). The contact and molecular interaction of somatic cells with each other is the main factor that enables stem cells to differentiate into haploid mature cells (12). In transplantation studies, it is seen how effective Sertoli cells and germ cells are in proliferation and differentiation. This is confirmed by the occurrence of spermatogenesis when normal Sertoli is transplanted to recipient infertile mutant testicles with Sertoli cell defects (16, 17).

Moreover, increasing the number of Sertoli cells in the recipient testis also increases niches (18). So we can say that disruption of communication between somatic cells and niche will naturally impair spermatogenesis. SCC niche performs proliferation and differentiation via endocrine and paracrine signals. Glial cell-derived neurotrophic factor (GDNF), one of the cytokines secreted by Sertoli cells and peritubular cells, plays a key role in this arrangement in the niche. GDNF provides SCC's proliferation by affecting surface receptor-complex of A single, A paired, A aligned cells (19). It has been shown that GDNF and fibroblast growth factor 2 (FGF2) are found in mouse Sertoli cells (20). Likewise, GDNF is also produced by peritubular myoid cells (21). The proliferation of SCCs is also provided by the cytokine colony-stimulating factor1 (CSF1) produced by peritubular myoid cells and Leydig cells in the interstitial area outside the tubules (22).

The tight junction complexes between Sertoli cells form the blood-testis barrier. This barrier divides the area of the seminiferous tubules into two parts as basal and adluminal compartments. The formation of these compartments plays an important role in the differentiation of germ cells. In addition, the blood-testis barrier provides a selective flow between interstitial fluid and adluminal fluid. This situation creates an immune-privileged environment for haploid germ cells in the adluminal compartment in the seminiferous tubules (23). Thus, identifying the details of SCC niche components in mammalian testicles is important to understanding the basis of sustained spermatogenesis.

1.2. Transplantation of Spermatogonial Stem Cells

Spermatogonial stem cell transplantation was first performed in 1994 by Brinster RL et al. (24). They isolated SCC from 4-12 day old mice, applied chemotherapy (busulfan), and transplanted into adult mice that developed azoospermia. They performed spermatogenesis in 70% of the recipients 35 days after transplantation. They found that the recipient testicular Sertoli cells were sufficient for structuring.

In ongoing studies, they obtained offspring eight months after transplantation of SCCs from mice of different age groups to the recipient (25). Spermatogenesis was performed with autologous orthotopic and ectopic transplantation of SCCs in primates (4). In monkeys, spermatozoa and embryos could be formed by autologous transplantation of SCCs (3).

In other ongoing studies, spermatogenesis was performed with the transplantation of SCCs in primates after freezing and thawing, and offspring could be obtained with these cells after three months, five months, and 99 days (26, 27, 28). Offspring could also be produced in some farm animals by donor transplantation (29, 30). However, problems such as the long time required for the cells to settle and become productive after transplantation, the low number of SCCs in the fresh testis, and the presence of numerical insufficiency in transplantation require the short-term culture of the SCCs (31). Since it is known that 4096 mature spermatozoa can be obtained (32) from a single SCC in rats where SCCs can reproduce numerically with the self-renewal feature (28, 33), fertility restoration requires at least 40-80 SCCs. In addition, the use of germinal stem cell lines seems appropriate in terms of solving the numerical problem.

Another problem is that the recipient testicle is older than the donor and requires more stem cells. Ensuring in vitro maturation of the SCCs in culture will solve the problems (5, 34). In addition, the application of Invitro maturation will eliminate the problem of contamination with cancer cells when the testicular tissue/suspension is transplanted.

1.3. Two-Dimensional Testis Cell Culture

In recent years, in vitro spermatogenesis of adult primate testicular cells has been attempted to achieve, but haploid cells could not be formed although it has reached the meiotic phase**.** Spermatocytes survived for about four weeks without separating into spermatids (35). Culture studies have also been conducted in adult men with maturation arrest for infertility treatment. Tesarik et al. reported that in vitro culture of adult human testicular cells with maturation arrest at the level of primary spermatocyte can be achieved spermatogenesis and can be obtained haploid cells and born alive baby by providing fertilization with these cells (36). In a similar situation, in vitro culture could reach the round spermatid stage (37). Lee et al. 2D cell cultures prepared from spermatogenic arrest and Sertoli cell-only in adult human testicles could reach microinjectable round spermatids (38). Spermatogenesis could not be completed in co-cultures with/without Leydig cells with Sertoli cells obtained from non-obstructive azoospermia adult human testicles (39). 2D culture media did not reflect the body environment, as the cells were grown in a single layer on plastic surfaces. Making 2D and, as an advanced stage, 3D cultures on brain-dead adult human germ cells K.Gholami et al. showed that 3D cultures were more efficient and successful in colony formation, proliferation, and differentiation than 2D culture (40). Prepubertal testicular cultures are seen as more complex applications compared to adult maturation arrest testicles. Prepubertal cultures are applications for the realization of two stages of spermatogenesis, including spermatocytogenesis and spermiogenesis. The small prepubertal testicular tissue and the small amount of SCC, the cells being in the gonocyte and spermatogonium stages, and the expectation that these cells perform both spermatocytogenesis and spermiogenesis reveal the complexity of the situation. The searches have been continued by carrying out various co-culture applications to provide the in vivo niche environment of adult mouse SCCs in vitro culture environment (41).

1.4. Tree-Dimensional Seminiferous Tubules Tissue / Organ Culture

It is necessary to develop testicular tissue culture and 3D culture research to overcome the deficiency of 2D cultures. When 3D culture was applied in a collagen matrix to adult human testicular cells with nonobstructive azoospermia with spermatocyte arrest, development was also achieved until the haploid round spermatid stage (42). With the applications made, moving to the advanced stage from spermatid was a serious problem in front of the researchers.

Sato T. et al. managed to overcome the challenge by developing a 3D in vitro culture method in the newborn mouse testis (43). They did in vitro organ culture of newborn mouse testis containing only gonocytes and primitive spermatogonia. They showed that spermatogenesis could occur in in vitro organ culture in more than two months. They were able to obtain spermatids and mature spermatozoa in testicular seminiferous tubules organ culture. They obtained offspring by microinjection with the produced spermatid and spermatozoa. This development positively contributed to elucidating the molecular mechanisms of spermatogenesis and the development of new diagnosis and treatment techniques in male infertility. They showed that spermatogenesis is possible even without in vivo circulation, and the method can be applied in mammals. Their ongoing studies proved that spermatogenesis error caused by microenvironment disorder in the original testis could be overcome by in vitro culture (44). They showed that by providing physiological conditions in mammals, spermatozoa could be obtained from germinal serial cells in about six weeks, and this culture can be applied (45). They stated that in vitro organ culture developed by adapting serum-free culture medium to old culture systems could be a simple and easily applicable method. They also developed spermatids and spermatozoa from freeze-thawed prepubertal testicular tissue by in vitro culture and obtained healthy offspring by microinjection. They showed that healthy offspring could be created with this method after treating childhood cancers (46, 47).

Komeya et al. developed this system a little further and adapted micro-fluid millipore membrane technology to seminiferous tubules organ culture (48). By creating a culture environment more suitable for physiological homeostasis, they provided spermatogenesis in the newborn mouse testis for a long period of six months. Thus, seminiferous tubules preserved their function in the culture environment for months. At the same time, they provided endocrine function by releasing luteinizing hormone and testosterone with this system. As a result, they obtained offspring with the formed spermatids and spermatozoa. Working differently, Michele et al. were able to preserve the integrity of the seminiferous

tubules by applying organotypic culture to human prepubertal testicular tissue (49). They saw that Sertoli cell maturation took place, and testosterone was produced in 139 days. However, a progressive loss of spermatogonium occurred, and differentiation into a haploid cell could not occur. Thus, the desired success could not be achieved. In human prepubertal testicles, failure to achieve in vitro spermatogenesis can be explained by the reasons such as the prepubertal process taking longer in humans compared to rodents, the need for different molecules, and the presence of unknown points about the niche.

1.5. Testicular Organoids

It is seen that the microenvironment must be provided in vitro for the SCCs to survive and transform into a haploid cell that will gain the ability to proliferate, differentiate and fertilize the oocyte (50). For in vitro maturation, Zhang et al. tried to reconstruct the testicles from newborn mouse testicular cells in a 3D culture medium (51). Although limited, they obtained seminiferous tubules formation and blood testicular barrier formation. They made the differentiation happen from spermatogonium to the primary spermatocyte stage. Mincheva M. et al. showed that somatic cells tend to form tubule-like structures in adult human in vitro primary testicular somatic cell culture and that Sertoli and myoid cells interact (52). 3D models have been useful in tracking the types of cellular interferences that induce in vitro cellular events and mechanisms. 3D models have been useful in tracking the mechanisms that create cellular interactions that induce these events in vitro.

In this area, an in vitro system is tried to be produced that fully models and mimics the testicular microenvironment and SCC niche. Organoids and organoid scaffolds may also be useful in in vitro 3D testicular tissue culture and providing the microenvironment to mimic the organism. Problems of SCC maturation can be overcome by using the testicular organoid augmentation method. Organoids are three-dimensional structures composed of multiple cell types that recapitulate the cellular architecture and functionality of natural organs. Organoids can be used as a physiologically feasible model system to study intercellular interaction, development, and tissue morphogenesis (7). In cell biology, the organoid niche can determine the epigenetic modification associated with signals, stem cell differentiation, and reprogramming of somatic cells. Organoids derived from stem cells are opening new avenues for modeling human diseases and regenerative medicine. However, many difficulties have to be overcome for stem cells to be applied versatilely in clinics. Stem cells, which build all the structural and functional units in the human body, can solve hematological, heart, nervous and digestive system diseases, cancer (53), and fertility restoration. With this in mind, some researchers working in male reproductive biology began to establish and characterize testicular organoids.

Alpes-Lopes et al. succeeded in creating a blood-testicular barrier by making 3D organoid culture from rat testicular cell suspension (8, 54). In addition, they developed the “Three Layer Gradient System”. This model contributed to the in vitro niche formation by showing the germ cell-somatic cell relationship and forming round tubular structures similar to the seminiferous tubules. It also created a new platform to investigate unknown factors in SCC proliferation and differentiation.

Pendergraft et al. used three adult human testicular tissues with brain death (55). They added a human testicular extracellular matrix to the culture medium. They added Sertoli, Leydig cells, which were obtained from the testis and immortalized, to the culture together with the SCCs. They obtained a testicular organoid. They were able to generate post-meiotic germ cells, albeit at a low rate. They provided a valuable model for germ cell biology. They have shown that human testicular organoids will be useful in the formation of the invitro niche.

On the other hand, Sakib et al. developed a pyramid-shaped microwells culture system suitable for the special architectural structure of the testicle (56, 57). They tried to create testicular organoids by placing prepubertal testicular cells in microwells by centrifugation. They created multicellular 3D testicular organoids from mammalian testicular tissues such as humans, pigs, mice, and monkeys. They showed that vitrified testicles could also be used to create testicular organoids. They showed that thousands of homogeneous organoids could be produced in this way.

Topraggaleh et al. produced testis-derived macropore scaffolds from mouse testicular extracellular matrix (58). They enabled the SCCs to differentiate until the post-meiotic period in culture. Furthermore, they showed that it could provide a new platform for testicular tissue engineering and in vitro spermatogenesis.

2. CONCLUSION

In general, 2D culture studies were able to reach the spermatid stage. Then, it is seen that spermatogenesis can be achieved in 3D cultures. However, the completion of in vitro meiosis and spermatogenesis, the production of haploid cells from SCCs under culture conditions, and the provision of niche seem to be a difficult practice. In vitro seminiferous tubules, tissue/organ culture, and testicular habitat maintenance require great skills and conditions to be overcome. Extracellular testicular matrix, testicular organoids, and organoid scaffolds supported with various substances appear to be a new and developing strategy for in vitro maturation. Organoid and organoid scaffolds will contribute to fertility restoration in prepubertal children and adult spermatogenic arrest, Sertoli cell-only patients. However, more work is needed to optimize the systems by developing and standardizing them with tissue engineering.

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