

Review

Spermatogonial Stem Cells: A Current Update in the Management of Azoospermic Infertile Men

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Abstract: One of the management of azoospermia is utilizing Assisted Reproductive Technologies (ART) by Intra-Cytoplasmic Sperm Injection (ICSI) with sperm obtained from the epididymis or directly from testis. In some conditions such as hypogonadism or undescended testis, the sperm are not successfully obtained. Thus, it is considered to perform Spermatogonial Stem Cells (SSCs) culture to restore men fertility. The objective of this work was to systematically review and discuss articles dealing with the subject of SSCs as a current update of azoospermic management. To this purpose, a computerized search of PubMed database was performed on the general term such as “male infertility”, “azoospermia” and “human spermatogonial stem cells” and the articles obtained were evaluated and collected. SSCs are adult stem cells which capable to self-renewal, differentiate, transmit the material genetic to offspring. SSCs have a vital role in spermatogenesis and in vitro proliferation which could be utilized for azoospermic management. Some alternative of SSCs utilization followed by ICSI in men with azoospermia, the formation of artificial sperms and auto-transplantation of in vitro proliferated SSCs, are under the preclinical phase of development. SSCs utilization is the proper approach to manage the azoospermic infertile men who sperm couldn't be obtained. The procedure of SSCs utilization such as culture method, including isolation, identification and tumor forming, should be investigated prior to clinical application.

Keywords: Male Infertility, Azoospermia, Spermatogonial Stem Cells

Introduction

Infertility has become a major reproductive health and social concern afflicting 10 to 15% couples worldwide, in which male infertility contributes ranging from 2.5 to 12% of the cases (Wang *et al.*, 2016; Cocuzza *et al.*, 2013). Male infertility is considered by the manifestation of some conditions such as congenital urogenital abnormalities, malignancy, urogenital tract infection, temperature increment in the scrotum (varicocele), genetic defects of the endocrine system, genetic disorders and immunological factors (Cocuzza *et al.*, 2013; Vlajkovic *et al.*, 2012). Male infertility could be observed and diagnosed by a low count of sperm (oligozoospermia), decreased sperm motility (asthenozoospermia), abnormal morphology (teratozoospermia) and the absence of sperm (azoospermia). Azoospermia is defined as the absence of sperm in the ejaculate which identified in approximately

10 to 15% of infertile males (Cocuzza *et al.*, 2013). One of the management of azoospermia is utilizing Assisted Reproductive Technologies (ART) by Intra-Cytoplasmic Sperm Injection (ICSI) which sperm retrieved from the epididymis by percutaneous/microsurgical epididymal sperm aspiration (PESA/MESA) or directly from testis by testicular sperm aspiration/extraction (TESA/TESE). In some conditions such as hypogonadism or undescended testis, the sperm are not successfully retrieved. Thus, it is considered to perform Spermatogonial Stem Cells (SSCs) to manage these infertile men (Goossens and Tournaye, 2014; Vassena *et al.*, 2015).

Spermatogonial Stem Cells as a Potential Management of Azoospermic Infertile Men

SSCs are adult stem cells which reside in the basal layer of the seminiferous tubules, surrounded by Sertoli cells. SSCs capable to self-renewal, differentiate,

transmit the material genetic to offspring. SSCs have a vital role in spermatogenesis by providing sperm production throughout life in men (Vassena *et al.*, 2015; Yang *et al.*, 2014). Recent studies have demonstrated that SSCs have a potential role in male infertility management (Wang *et al.*, 2016). However, a rarity and limitation of the specific biomarker of SSCs could be one of significant challenge in SSCs utilization (Yeh *et al.*, 2009). Recently, SSCs utilization followed by ICSI in infertile men, the formation of artificial sperms and auto-transplantation of in vitro proliferated SSCs, are in under the preclinical phase of development. The optimum development of culture technique and auto-transplantation of SSCs would have enormous benefits, especially for the management of azoospermic infertile men.

One of the management of azoospermia is utilizing ARTs by ICSI which sperm obtained from the epididymis (PESA/MESA) or directly from testis (TESA/TESE), as long as spermatogenesis occurs. However, male infertile patients caused by hypogonadism or undescended testis, the sperm are not successfully obtained. Thus, it is considered to perform SSCs (Goossens and Tournaye, 2014; Sadri-Ardekani *et al.*, 2014). Recently, there are some possibility of SSCs utilization followed by ICSI in male infertility management such as artificial sperms formation by culture and auto-transplantation of in vitro proliferated SSCs which under preclinical phase of development.

Culture Systems of In Vitro Germ Cell

Several culture systems have been established in order to conduct optimal in vitro germ cell culture system which could maintain SSCs pluripotency or achieve sperm maturation as demonstrated in Table 1. The optimum condition of SSCs culture system is required in order to achieve in vitro spermatogenesis which might be used in ART. Three-dimension culture systems have been developed such as matrigel (Choi *et al.*, 2014) and hydrogel (Guo *et al.*, 2015) which could

supports proliferation and long term maintenance of SSCs. Additionally, culture systems using coating plate (Koruji *et al.*, 2012; Sadri-Ardekani *et al.*, 2014; Goharbakhsh *et al.*, 2013; Lim *et al.*, 2010) and co-culturing SSCs with somatic testicular tissue such as Sertoli cell (Zanganeh *et al.*, 2013; Mohamadi *et al.*, 2012; Rastegar *et al.*, 2015; Liu *et al.*, 2011; Akhondi *et al.*, 2013) have been developed in order to maintain SSCs pluripotency. Elhija *et al.* (2011) studied about producing post meiotic germ cells including morphologically normal sperm which retrieved from testicular cells of immature mice in a soft agar culture system. Briefly, three research groups conducted the establishment of germ cells derived from mouse embryonic stem cells (Aponte *et al.*, 2013). Geijsen *et al.* (2004) investigated in producing gamete-like cells from mouse embryo stem cells and using ICSI as a functional assay. Thus, embryo was obtained after fertilization and reached the blastocyst stage. The other research (Nayernia *et al.*, 2006; Toyooka *et al.*, 2003) studied in generating sperm in vitro, but only one of them using ICSI as a functional assay to obtain offspring. However, the low efficiency outcome was generated and the mice died prematurely (Nayernia *et al.*, 2006). Recently, in vitro spermatogenesis from human SSCs has not been developed yet, even though in vitro spermatogenesis has been successfully conducted in rodents resulting in the healthy offspring after ICSI (Goossens and Tournaye, 2014).

The authors assessed that the isolation, culture, expansion, and identification technique of SSCs should be optimized in order to make SSCs feasible in research and clinical medicine. Some limitations still restrain the well-development of SSCs culture system. As has known various factors could influence SSCs culture condition such as conditioned-medium, serum and feeder cells utilization, hormonal treatment. An effort in order to mimic the microenvironment of the testes to in vitro testicular model has been a challenge in growing sperm successfully.

Table 1: In-vitro germ cell culture system

Spermatogonial Stem Cells Culture	Reference
Development of three-dimension culture systems to support proliferation and long term maintenance of SSCs.	Choi <i>et al.</i> (2014); Guo <i>et al.</i> , (2015).
Development of coating plate culture systems which capable to support the proliferation and self-renewal of SSCs.	Koruji <i>et al.</i> (2012); Ardekani <i>et al.</i> , 2009; Goharbakhsh <i>et al.</i> , 2013; Lim <i>et al.</i> , 2010).
Co-culturing SSCs with somatic testicular tissue such as Sertoli cell as feeder layer could support proliferation and differentiation of SSCs.	Zanganeh <i>et al.</i> (2013); Mohamadi <i>et al.</i> , 2012; Rastegar <i>et al.</i> , 2015; Liu <i>et al.</i> , 2011; Akhondi <i>et al.</i> , 2013).
Expansion and differentiation of testicular cells from 7-day-old immature mice in a soft agar culture system, thus post meiotic germ cells were produced including morphologically normal sperm.	Elhija <i>et al.</i> (2011).
Producing gamete-like cells from mouse embryo stem cells and using ICSI as a functional assay. Embryo was obtained after fertilization and had reached the blastocyst stage.	Geijsen <i>et al.</i> (2004).
Generating mouse sperm in vitro and using ICSI as a functional assay to obtain offspring.	Nayernia <i>et al.</i> (2006).
Generating sperm <i>in vitro</i> from mouse embryo stem cells.	Toyooka <i>et al.</i> (2003).

Nevertheless, obtaining sufficient SSCs clinical utilization is crucial, therefore establishment of a stable culture system which could maintain propagation of SSCs in vitro is required. In the future decade, authors believed that developing the stable culture system of SSCs will be an attainable goal, yet the time for the successful in vitro differentiation is less predictable.

Spermatogonial Stem Cells Transplantation

Several researches of SSCs transplantation have been conducted in which used as a functional assay and potential fertility men restoration possibility as shown in Table 2. SSCs transplantation is suggested could contribute to re-establish men fertility by increasing in vivo spermatogenesis possibility followed by natural conception achievement or ARTs by ICSI. Breakthrough of SSCs transplantation by Brinster and Zimmermann (1994) inspired the onset of testicular stem cell research in which became the cornerstone of germ line stem cell utilization. They investigated SSCs transplantation which derived from wild type mouse into the testes of chemotherapy-treated mice to restore spermatogenesis. In the recipient testes, the transplanted SSCs showed capability to expand and differentiate forming mature sperm and restore spermatogenesis. The result showed that donor SSCs could engraft in testes, re-establish spermatogenesis, thus leading to the offspring production. Human SSCs auto-transplantation has been conducted firstly by Radford *et al.* (1999) according to recent scientific literature. They introduced cryopreservation and auto-transplantation human SSCs from 12 male non-Hodgkin's lymphoma patients who underwent chemotherapy. Seven patients had the cells re-infused back into their testes but the outcome is remain unknown because there have been no follow-up reports on the fertility status. Recently, Choi *et al.* (2014) developed a three dimension of culture system for long term culture of mouse SSCs and SSCs functionality was confirmed by transplanting in vitro proliferated mouse SSCs to chemotherapy-treated mice. Whilst Sadri-Ardekani *et al.* (2009) and Koruji *et al.* (2012) transplanted human SSCs into the seminiferous

tubules of chemotherapy -treated mice, hence the presence of transplanted cells was detected in the basal compartment of the seminiferous tubules in the recipient testes after xenotransplantation. The authors assessed according to published reports that transplanting in vitro proliferated SSCs into recipient could be used to receive functional sperm in which capable of producing fertile offspring. SSCs transplantation has several benefits such as allowing the possibility to auto-transplantation in human which could allow natural conception. Even though SSCs transplantation in mice and other higher mammals has been successful, several considerations still restrain the application of this technology in humans (Vassena *et al.*, 2015; Valli *et al.*, 2014).

Some limitations upward ahead are the limited number of SSCs which could be obtained is suggested not sufficient enough to recolonize and restore fertility, hence it should be propagated in vitro previously. Furthermore, auto-transplantation of SSCs is a remaining risk of tumor forming and invasive procedure requirement is needed. Additionally, the health quality of offspring including genetic and epigenetic alteration has not been extensively studied yet (Liu *et al.*, 2011). Nevertheless, based on previously research, there is a chance and it should be possible to transplant testicular tissue or cells, as the potential management of azoospermic man, for regeneration of spermatogenesis in the future time.

The Challenge of SSCs Utilization

The authors addressed that there are some challenges up forward regarding to utilization of SSCs in clinical application as presented in Table 3. Like another adult stem cells, SSCs has a capability of unlimited proliferation, there is a possibility leading to tumor formation if its characteristic was altered (Kubota *et al.*, 2006). Another consideration, transplantation of *in vitro* proliferated SSCs culture could be contaminated with tumor cells, as well as the un-growth cells, thus also driving to tumor formation, even there were several methods to identify the SSCs yet the possibilities still remained.

Table 2: The transplantation of spermatogonial stem cells

The Transplantation of Spermatogonial Stem Cells	Reference
Transplantation of mouse SSCs into the chemotherapy-treated mice. Donor SSCs could engraft and re-establish spermatogenesis, thus leading to the offspring production.	Brinster and Zimmermann (1994; Avarbock <i>et al.</i> , 1996).
Introducing cryopreservation and human SSCs auto-transplantation from 12 male non-Hodgkin's lymphoma patients who underwent chemotherapy. Seven patients had the cells injected back into their testes, yet the outcome is unknown.	Radford <i>et al.</i> (1999).
Transplantation of mouse SSCs into the chemotherapy-treated mice. SSCs was confirmed and found in the recipient testicular tubules.	Choi <i>et al.</i> (2014).
Xenotransplantation of human SSCs into the seminiferous tubules of chemotherapy -treated mice. The presence of transplanted cells was detected in the basal compartment of the seminiferous tubules in recipient testes.	Koruji <i>et al.</i> (2012; Sadri-Ardekani <i>et al.</i> , 2009).

Table 3: The challenge of spermatogonial stem cells utilization

The Challenge of Spermatogonial Stem Cells Utilization	Reference
Defining a method and culture system	Gassei and Orwig (2016)
Possibility of epigenetic or genetic alteration	Samplaski <i>et al.</i> (2014)
Possibility of tumor formation	Kubota and Brinster (2006)
Should be increased in number of SSCs prior to utilization	Samplaski <i>et al.</i> (2014)
Functionality and mechanism of SSCs in regenerating spermatogenesis	Gassei and Orwig (2016)
Ethical concern	Kubota and Brinster (2006)

Generally the number of SSCs obtained usually in limited number, it should be propagate for obtaining an adequate amount of cells in advance of auto-transplantation to the patient. Nevertheless, in vitro culture system still could not assure the integrity of genetic material of SSCs yet. There is little research conducted on any genetic and epigenetic alteration in either the transplanted SSCs or offspring (Samplaski *et al.*, 2014). During cells propagation through culture, it is possible in occurring of epigenetic or genetic alteration although the offspring obtained seen to be healthy in animal models. Exposure of growth factor, serum and the maturation processes contribute to genetically modification. Particularly, male germ cells undergo epigenetic remodeling during spermatogenesis, errors during the process may cause altered genetic information to be transmitted to offspring generating in abnormalities. The health quality of offspring could be affected, as of the genetic and epigenetic screening of the cells is required in advance of human SSCs utilization in clinical application.

There is not gold standard in order to measure the functionality of human SSCs, even transplantation to restore spermatogenesis with functional sperm and offspring in animal model already conducted (Gassei and Orwig, 2016). Nowadays, the successful of generating offspring through SSCs transplantation could be observed in mice, rat and other mammals, but there is an ethical consideration of stem cell based therapy especially SSCs in human. Another interest point under investigation is the mechanism of SSCs in re-establish spermatogenesis, as well as another kind of stem cells, whether the injected cells could proliferate or the paracrine factor which suggested stimulate the reside stem cells is remain unknown. Despite many challenges ahead, SSCs utilization is one of promising agent which could be used as potential management of azoospermic men in the future.

Conclusion

In conclusion, after reviewing the recent scientific literature, to date, even though there are no stem cell based therapies which available, some of the clinical trials are being held to ameliorate or solve the reproductive medicine issues such as male infertility. SSCs utilization is the proper approach to manage the azoospermic infertile men whose sperm couldn't be retrieved. *In vitro* production of sperm and autologous transplantation are currently under clinical trial phase

development. Surely, the procedure of SSCs utilization such as culture method including isolation, purification, identification, and functional assay as well as possible tumor formation should be investigated prior to clinical application.

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Authors' Contribution

Silvia W Lestari: First author of the manuscript, journal collection and supervisory role of the review article.

Debby Aditya: Journal collection and manuscript review article.

Ethics

This review article is original and contains unpublished material. The corresponding author confirms that all of the authors have read and approved the manuscript and no ethical issues involved.

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