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RESEARCH ARTICLE

Advances in spermatogonial stem cell research: A systematic review and bibliometric analysis

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ABSTRACT

Spermatogonial stem cells (SSC) are an exceptionally rare group of testicular cells that give rise to all subsequent male germ cells, the basis of spermatogenesis and male fertility. The SSC have shown great potential in livestock breeding systems, medical applications, and genetic conservation of endangered species. In this study, we used bibliometric analysis to assess worldwide research trends in the SSC area based on publication outputs, co-authorships among authors, and affiliated countries. A systematic review was performed to discuss: 1) The culture system of SSC, 2) The methods used to generate an ideal male for SSC transplantation, 3) The type of transplantation and the success of SSC transplantation in various species, 4) Application of SSC culture and transplantation technique to agricultural practice. Results of the bibliometric analysis on the Scopus database showed a total of 2171 research articles issued between 1965 to 2023. With a consistent annual publication of more than 100 articles, it was suggested that a strong interest in SSC research started in the last 11 years. About 54% of the total global publications were contributed by researchers from the United States of America (USA) and China, leading the other 59 countries/territories. The three main topics in SSC research are the culture system, generation of an ideal male recipient, and SSC transplantation applications. The culture system has the highest number of publications, reflecting its importance in studying and manipulating SSC. Currently, several challenges must be addressed before this technique can be applied in agricultural contexts, particularly the establishment of a stable long-term culture system. Looking ahead, we anticipate ongoing development and optimization of the SSC culture system and its application in the field, which will enhance valuable approaches for improving livestock production.

1. INTRODUCTION

Spermatogonial stem cells (SSC) found in the testes are responsible for spermatogenesis and male fertility (Phillips et al., 2010). SSC have the following essential characteristics, i.e., it could undergo self-renewal to sustain the stem cell pool and undergo differentiation to sustain constant sperm production in post-pubertal males, similar to other stem cells (De Rooij and Grootegoed, 1998; Fayomi and Orwig, 2018). The SSC are rare, comprising 0.03% of total germ cells in the testis of mice (Fayomi and Orwig, 2018; Tagelenbosch and De Rooij, 1993). Understanding the cell engenders new opportunities for developing novel technologies, such as SSC transplantation. Spermatogonial stem cell transplantation (SSCT) is a technique developed and first introduced by Brinster and colleagues almost three decades ago (Brinster and Zimmermann, 1994). This technique consists of the isolation of SSC from a donor animal, in vitro expansion of the cell, and the transplantation of these cells into a recipient testis, where these cells will grow and mature into sperm cells carrying the genetic features of the donor. Research on SSC holds significant promise for regenerative medicine and treating male infertility. Techniques such as SSC transplantation and in vitro propagation can potentially restore fertility in males. Despite several studies on SSC over the last few years, its practical applicability remains limited. We offer a systematic review and bibliometric analysis on this topic to help address the main scientific challenges of its practical applications. The aim of this study is to comprehensively examines the current status and the future of this field through a detailed review and large-scale bibliometric analysis.

2. MATERIALS AND METHODS

2.1 Systematic review

The systematic review was conducted to discuss the following:

a) The culture system of SSC.
b) The methods used to generate an ideal male for SSC

transplantation.

c) The type of transplantation and the success of SSC transplantation in various species.

d) Applications of SSC culture and transplantation techniques to agricultural practices.

2.2. Bibliometric analysis

2.2.1 Data source and the search strategy

Data mining was conducted using the Scopus database between March 17 and 21, 2024. The study's primary focus is on research publications using the phrase "spermatogonial stem cell" in the title and abstract. The dataset spanned from the first published article in 1965 to the most recent in 2023. The query string used for the search was: (TITLE-ABS ("spermatogonial stem cell")) AND (EXCLUDE (PUBYEAR, 2024)) AND (LIMIT-TO (DOCTYPE, "ar")) AND (LIMIT-TO (SRCTYPE, "j")).

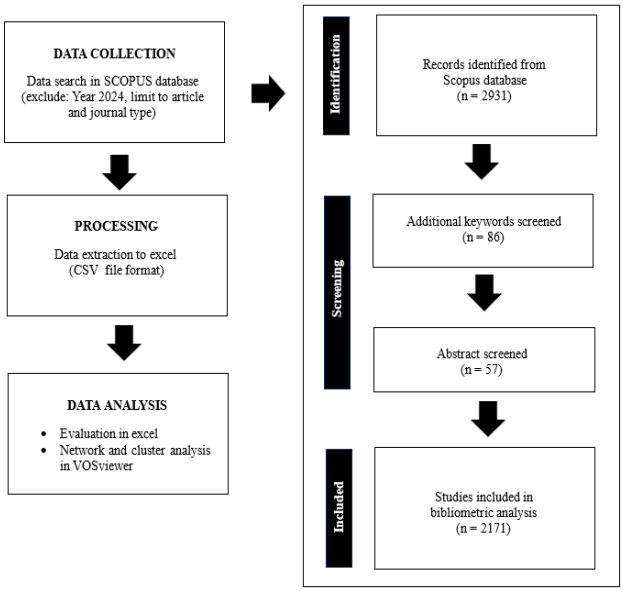


Figure 1: Flow chart of method and literature collecting.

Approximately 2171 documents were retrieved using the query string. Additional keywords such as review, recent, progress, highlight, revisit, advance, critical in the title, and abstract were added to the query string to ensure that no review articles were included in our study, resulting in 86 possible irrelevant articles. After screening the abstracts and full texts, 57 were published as review articles. The Scopus unique article identification (EIDs) of these review articles were used to exclude the identified review publications from the final bibliometric analysis.

2.2.2 The bibliometric maps

The data was exported in the .csv file format for analysis in Microsoft Excel and VOSviewer. VOSviewer (version 1.6.17), a bibliometric mapping and visualization software, was used to create and visualize the bibliometric networks. The bibliometric analysis explored both Excel and VOSviewer for both the quantitative and qualitative. VOSviewer is an open-source software for network and visualization analysis. Data cleaning in excel was performed to remove duplicates, messy data, and transforming formats. The graphs/tables and the maps contain items were generated in Microsoft Excel using the output data from VOSviewer.

In this study, the items are the objects of interest, namely the countries. Between any pair of items there can be a link connection or relation between two items. Each link has a strength, represented by a positive numerical value. The higher this value, the stronger the link. In the case of co-authorship analysis, the link strength between countries indicates the number of publications that two affiliated countries have co-authored, whereas the total link strength indicates the total strength of the co-authorship links of a given country with other countries. The closer two countries are located to each other in VOSviewer, the stronger their relatedness (co-authorship of publications). Also, the thicker the line connecting the two countries, the greater the number of documents they co-authored.

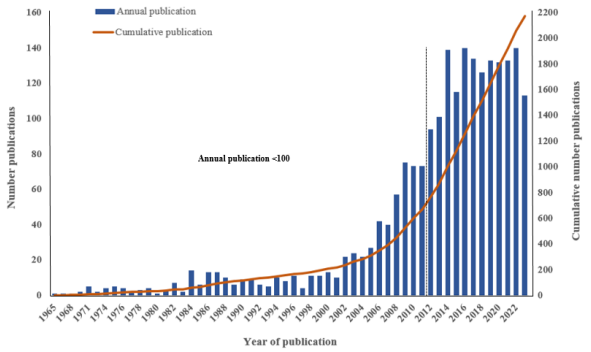


Figure 2: The annual and cumulative numbers of publications on SSC indexed in Scopus from 1965 to 2023.

3. RESULT AND DISCUSSION

3.1 Global research trends on SSC

The analysis results yielded a total of 2931 publications over 59 years. Of these, 2171 (74%) were research articles, while the remaining 26% were other types of publications, including reviews, book chapters, conference papers, erratum, short surveys, notes, editorial, books, letters, and data paper. The results of the bibliometric analyses on the 2171 research articles are shown in Figure 2. The oldest publication dates back to 1965. Although the annual publication has increased over the years, it has not been inconsistent. The annual publication recorded remained below 100 until 2013, was after which it consistently surpassed 100 per year. This indicates that SSC research

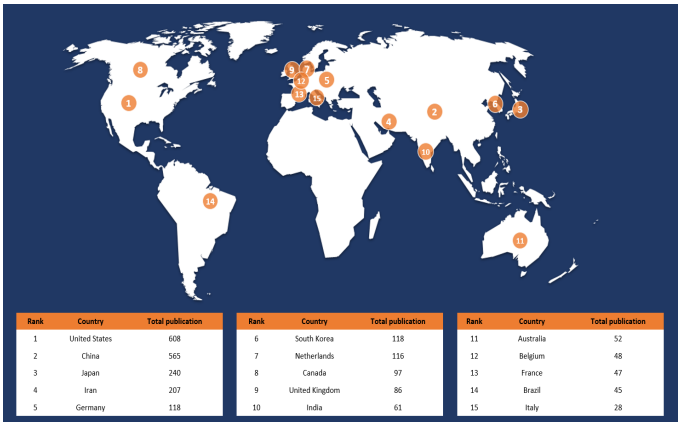
has gained significant attention of researchers over the past 11 years. The rising trend in publications within the SSC field signifies rapid advancements and expanding opportunities across various domains, from basic science and clinical applications to biotechnology and ethical discussions. This progress holds the potential to revolutionize our understanding and treatment of male infertility, contribute to livestock breeding, and prompt important ethical and regulatory considerations. Greater research efforts can lead to improved treatments for male infertility. This includes developing methods to culture and expand SSC in vitro, enabling autologous transplantation to restore fertility in men who have lost their germ cells due to medical treatments or genetic conditions. In agriculture, advancements in SSC research can enhance breeding programs, allowing for the propagation of desirable traits in livestock more efficiently. An increasing number of studies may prompt the development of more comprehensive regulatory frameworks to ensure ethical and safe application of SSC technologies.

Table 1 shows the top ten most productive journals by six publishers. The most productive journal in the SSC research field is the Biology of Reproduction by Elsevier, with a total publication of 153. The top-cited article (total citation: 1077) about SSC is the study by Braydich-Stolle et al. (2005), who investigated the suitability of a mouse SSC line as a model to assess nanotoxicity in the male germline (Table 2). Kanatsu-Shinohara (2003) also successfully established a long-term SSC culture of mice, with 861 total citations, rendering it the top two most cited articles on SSC studies. The rest of the top cited articles focused on establishing SSC lines by identifying spermatogonia-specific transcription factors, growth factors, or surface markers that require important understanding to regulate self-renewal and maintenance of the stem cell pool. The detailed procedure of SSC transplantation in mice is a prominent topic of interest among researchers, with a total citation of 515 (eight place).

Table 3 lists the top 15 leading authors in SSC, affiliated with 7 countries, as follows: the USA (5 authors), Japan (4 authors), Iran (2 authors), China (2 authors), Canada (1 author) and South Korea (1 author). The authors' affiliations indicated that SSC research is mainly associated with the medical field (five authors). The top two prolific authors are from Japan and affiliated with the same university, the Graduate School of Medicine, Kyoto University. Shinohara led the list with a record of 84 total publication, 41 h-index, and 6641 times citations, followed by Brinster and Avarbock, both affiliated with the University of Pennsylvania School of Veterinary Medicine, Philadelphia.

Figure 3 depicts the top 15 most prolific countries regarding SSC research activities. The USA and China produced around 54% of worldwide papers, suggesting that these two nations are major players in SSC research. With 608 publications, the USA is the most prolific country, accounting for 28% of all publications worldwide. China came second, with just 26% fewer publications than the USA. Although the top two prolific writers are from Japan (Table 3), the nation is ranked third, with 240 total articles.

Figure 4 depicts the distribution of countries/territories per region. The results of co-authorship indicated that the USA is the most affiliated country, with 262 occurrences of co-authorship, with 38 countries/territories. This is followed by the United Kingdom (UK, 22 links, 59 co-authorships), China (20 links, 98 co-authorships), Germany (19 links, 67 co-authorships), the Netherlands (18 links, 48 co-authorships), Japan (17 links, 49 co-authorships), and others. Meanwhile, about 49% of the listed countries had international collaborative publications with less than 10 countries. Furthermore, in the SSC article publication, five countries (Bosnia, Poland, Jordan, Nigeria, and Peru) are not linked to any country.



Rank	Country	Total publication
1	United States	608
2	China	565
3	Japan	240
4	Iran	207
5	Germany	118
6	South Korea	118
7	Netherlands	116
8	Canada	97
9	United Kingdom	86
10	India	61
11	Australia	52
12	Belgium	48
13	France	47
14	Brazil	45
15	Italy	28

Figure 3: The top 15 most productive countries in SSC publications.

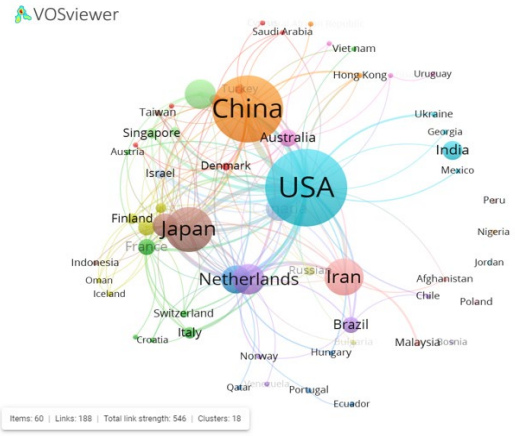


Figure 4: A screenshot of the bibliometric map created based on co-authorships with network visualization mode, VOSviewer URL: https://bit.ly/3z1K6d9

Table 1: The top ten most productive journals on SSC research with their most cited article.

Rank	Journal	Total publication	CiteScore 2022	SJR 2022	SNIP 2022	Percentile	Quartile	Title of the most cited article	Times cited	FWCI	Publisher
1	Biology of Reproduction	153	6.5	0.991	0.959	90%	Q1	Long-term proliferation in culture and germline transmission of mouse male germline stem cells	861	5.77	Elsevier
2	Plos One	56	6.0	0.885	1.253	87%	Q1	Spermatogonial stem cell niche and spermatogonial stem cell transplantation in zebrafish	136	2.19	Public Library of Science
3	Mutation Research Fundamental and Molecular Mechanisms of Mutagenesis	49	5.7	0.111	0.650	82%	Q1	A quantitative study of spermatogonial multiplication and stem cell renewal in the C3H/101 F1 hybrid mouse	486	0.77	Elsevier
4	Human Reproduction	44	10.5	1.711	1.929	95%	Q1	Restoration of fertility in infertile mice by transplantation of cryopreserved male germline stem cells	144	4.10	Oxford University Press
5	Theriogenology	42	5.6	0.764	1.186	91%	Q1	Basic features of bovine spermatogonial culture and effects of glial cell line-derived neurotrophic factor	84	2.73	Elsevier
6	Proceedings of The National Academy of Sciences of The United States of America	40	19.2	4.026	2.765	94%	Q1	Growth factors essential for self-renewal and expansion of mouse spermatogonial stem cells	797	3.81	National Academy of Sciences
7	Reproduction	38	7.3	0.989	1.019	76%	Q1	Isolation and purification of type A spermatogonia from the bovine testis	178	1.17	BioScientifica Ltd.
8	Scientific Reports	35	7.5	0.973	1.312	92%	Q1	Generation of germline ablated male pigs by CRISPR/Cas9 editing of the NANOS2 gene	88	2.87	Springer Nature
9	Stem Cell Reports	32	11.6	2.464	1.209	91%	Q1	Functional differences between GDNF-dependent and FGF2-dependent mouse spermatogonial stem cell self-renewal	131	4.38	Elsevier
10	Animal Reproduction Science	30	4.2	0.559	1.041	92%	Q1	Germ cell transplantation and testis tissue xenografting in domestic animals	59	3.17	Elsevier

Table 2: The ten most cited manuscript on SSC studies.

No	Article	Author	Journal name	Year published	Total citation
1	In vitro cytotoxicity of nanoparticles in mammalian germline stem cells	Braydich-Stolle L., Hussain S., Schlager J.J., Hofmann M.-C.	Toxicological Sciences	2005	1077
2	Long-term proliferation in culture and germline transmission of mouse male germline stem cells	Kanatsu-Shinohara, M., Ogonuki, N., Inoue, K., Miki H., Ogura A., Toyokuni, S., Shinohara, T.	Biology of Reproduction	2003	861
3	Growth factors essential for self-renewal and expansion of mouse spermatogonial stem cells	Kubota, H., Avarbock, M.R., Brinster, R.L.	Proceedings of the National Academy of Sciences of the United States of America	2004	798
4	Essential role of Plzf in maintenance of spermatogonial stem cells	Costoya, J.A., Hobbs, R.M., Barna, M., Cattoretti G., Manova K., Sukhwani M., Orwig K.E., Wolgemuth, D.J., Pandolfi, P.P.	Nature Genetics	2004	791
5	Pluripotency of spermatogonial stem cells from adult mouse testis	Guan, K., Nayernia, K., Maier, L.S., Wagner S., Dressel R., Jae H.L., Nolte J., Wolf F., Li M., Engel, W., Hasenfuss, G.	Nature	2006	757
6	In vitro production of functional sperm in cultured neonatal mouse testes	Sato, T., Katagiri, K., Gohbara, A., Ogonuki N., Ogura A., Kubota, Y., Ogawa, T.	Nature	2011	569
7	β1- and α6-integrin are surface markers on mouse spermatogonial stem cells	Shinohara, T., Avarbock, M.R., Brinster, R.L.	Proceedings of the National Academy of Sciences of the United States of America	1999	516
8	Transplantation of testis germinal cells into mouse seminiferous tubules	Ogawa, T., Aréchaga, J.M., Avarbock, M.R., Brinster, R.L.	International Journal of Developmental Biology	1997	515
9	A quantitative study of spermatogonial multiplication and stem cell renewal in the C3H/101 F1 hybrid mouse	Tagelenbosch, R.A.J., de Rooij, D.G.	Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis	1993	486
10	In Vitro-Differentiated Embryonic Stem Cells Give Rise to Male Gametes that Can Generate Offspring Mice	Nayernia, K., Nolte, J., Michelmann, H.W., Lee J.H., Rathsack K., Drusenheimer N., Dev A., Wulf G., Ehrmann I.E., Elliott D.J., Okpanyi V., Zechner U., Meinhardt, A., Engel, W.	Developmental Cell	2006	447

Table 3: List of the 15 most prolific authors in the SSC research

Rank	Author	Scopus author ID	Year of 1st publication	Total publication	Document h-index	Total citation	Current affiliation	Country
1	Shinohara, T.	7202958344	1999	84	41	6641	Graduate School of Medicine, Kyoto University	Japan
2	Kanatsu-Shinohara, M.	6603287818	2003	76	37	5140	Graduate School of Medicine, Kyoto University	Japan
3	Brinster, R.L.	7101751075	1996	53	44	9902	University of Pennsylvania School of Veterinary Medicine, Philadelphia	United States
4	Avarbock, M.R.	6701891909	1996	46	41	9021	University of Pennsylvania School of Veterinary Medicine, Philadelphia	United States
5	Orwig, K.E.	6604065476	2000	46	29	4359	Magee-Womens Research Institute, Pittsburgh	United States
6	Movahedin, M.	6507295983	2007	45	13	502	Tarbiat Modares University, Tehran	Iran
7	Oatley, J.M.	6602612889	2002	44	32	3743	Washington State University Pullman, Pullman	United States
8	Ryu, B.Y.	7005102179	2001	44	20	2068	Chung-Ang University, Seoul	South Korea
9	Ogura, A.	7101788606	2003	40	25	4098	Faculty of Medicine, The University of Tokyo, Tokyo	Japan
10	Ogonuki, N.	6701731717	2003	38	24	3947	Riken BioResource Research Center, Tsukuba	Japan
11	Dobrinski, I.	7003715886	1998	37	20	3057	University of Calgary, Calgary	Canada
12	Koruji, M.	23467323100	2009	35	17	935	Iran University of Medical Sciences, Tehran	Iran
13	He, Z.	8973145600	2007	34	20	1494	Hunan Normal University, Changsha	China
14	Li, B.	14033284200	2010	34	11	309	Yangzhou University, Yangzhou	China
15	Kim, B.J.	36468451900	2012	32	13	545	Columbia University Irving Medical Centre, New York	United States

3.2 Research trends of SSC publications based on the main topic

This paper reviewed three main topics in SSC research trends, including the culture system, the technique of generating an ideal male recipient, and the application of SSC transplantation (Figure 5). Our results show that among the three topics, the generation of the male recipient is the oldest study associated with SSC. The first study recorded was on the irradiation effect of SSC (Oakberg, 1971). The first study on the male recipient generation for the SSC transplantation technique was recorded in 1998. These findings indicate that the importance of depleting the endogenous germ cells in a recipient's testis for the application of SSC transplantation technology has been realized by earlier researchers. Although the first study was conducted almost 55 years ago, the total publication on this topic was the lowest (812), and the study on this topic was consistently low than the other two topics.

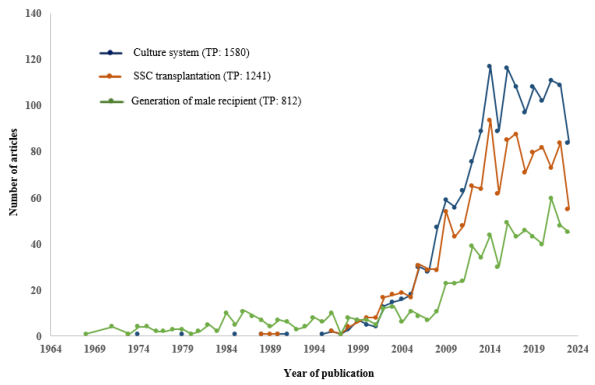


Figure 5: Research trends of the selected major topics in SSC (TP: total publication).

The topic associated with the SSC culture system recorded the highest total publications (1580) and showed an increasing trend throughout the years. This suggests the importance of cell culture system studies in the SSC research area. The development of cell culture technologies enables researchers to study SSC characteristics and expand and manipulate this rare cell. Most attention was given to this area as SSC culture due to its role in transplantation applications. While the application of SSC transplantation is imperial, with the highest publication until 2007, the attention was shifted to establishing the SSC culture system from 2008 until the present.

The development of long-term SSC culture is of utmost importance due to the challenges in recreating a suitable environment or an effective stem cell culture system. Nonetheless, the challenge has not been overcome due to the complexity of the "niche" required for the growth and expansion of SSC in a culture system. Sertoli, peritubular myoid, Leydig, and other interstitial cells in the testes contribute to the niche of SSC. However, the enzymatic treatment used in cell culture for tissue dissociation and cell isolation often affects the microenvironment (Aponte, 2015). Therefore, developing a niche that requires species-specific knowledge of SSC in an in vitro condition that mimics the in vivo environment is vital to preserving the functional characteristics of SSC.

3.2.1 SSC culture system

In vitro SSC culture is a crucial approach for extending and manipulating this uncommon cell population, and a long-term SSC culture is regarded as a requirement for in vitro investigation of SSC self-renewal and differentiation. To date, long-term SSC cultures of mice, rats, and hamsters have been established (Kanatsu-Shinohara et al., 2003, 2008; Kubota et al., 2004; Ryu et al., 2004). On the contrary, long-term cultivation of SSC from non-rodent species remains a challenge. Researchers can only sustain in vitro SSC proliferation for a short while for several years (no longer than 2 months; (Bahadorani et al., 2012; Fujihara et al., 2011; Heidari et al., 2012; Kadam et al., 2013; Kala et al., 2012; Nasiri et al., 2012; Xie et al., 2010; P. Zhang et al., 2017). Several studies have demonstrated that putative testicular SSC in domestic animals can be isolated and primarily cultivated easily. However, putative SSC proliferation gradually decreases during subculture, and differentiation and apoptosis increasingly dominate cellular events over time, halting SSC propagation. Large animal SSC may have special features since a long-term culture method built for rodent SSC has not been able to sustain the continuous proliferation of domestic SSC.

Methods to imitate the SSC niche in vitro and promote SSC development are being researched, including the use of nanofiber scaffolds that successfully mimic the structure of the body's original extracellular matrix (ECM), providing a good simulation of an in vivo environment (Shams et al., 2017) and optimizing the culture environment to favor glycolysis as an energy source (Helsel et al., 2017). Establishing a long-term culture of SSC from domestic animals in the near future is not unrealistic, as an establishment of an immortalized SSC line was accomplished in humans (Hou et al., 2015) and pigs (Zheng et al., 2020) recently.

3.2.2 Generation of an ideal male recipient of the SSC transplantation

Establishing an SSC culture system allows researchers to address the basic biological questions of SSC biology and open new possibilities for male germline manipulation. The potential application of SSC transplantation in agricultural, clinical, and wildlife conservation has given prominence to developing a sterile male to support donor-derived spermatogenesis following transplantation. Commercial application of SSC transplantation relies on a supply of males lacking germline stem cells. Thus, the preparation of sterile male or germ cell-depleted recipients is an essential step in spermatogonial stem cell transplantation (SSCT). The male recipient must be ascertained void of spermatogenesis, thereby ensuring that the donor germ cells can penetrate the epithelial layer of the Sertoli cells more easily and enter the basal lamina surrounded by the peritubular myoid cells. This is one of the key conditions for recipient testis to be successfully colonized by germ cell transplantation. Various methods have been used to generate evacuated testis to prepare recipients for SSCT, such as chemical treatment, irradiation, and gene editing.

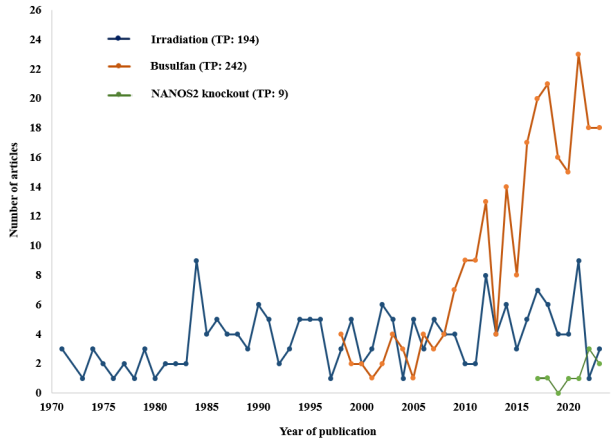


Figure 6: Research trends of the different generation methods of the ideal male recipient of SSCT. (TP: total publication)

SSC was not associated with means other than irradiation prior to 1998 (Figure 5). Earlier studies focused on understanding the SSC response to irradiation by evaluating the genetic damage, duration of the sterility, and SSC survival rate after radiation exposure. The benefits of the irradiation method in the SSC transplantation technique were only realized in early 2000. The first study recorded on the irradiation method on SSC transplantation technique was done by Dobrinski et al. (2001). From 1998 to 2009, publication trends are inconsistent between irradiation and busulfan. However, from 2010, reports of busulfan treatments consistently dominate the publication trends until present (2023).

Busulfan (1,4-butanediol dimethanesulfonate), a chemotherapeutic drug, is often used to deplete endogenous SSC by causing apoptosis of the germ cell population (Chen et al., 2018). However, busulfan has a narrow safety margin and causes harmful effects on animals. Busulfan administered in pigs and sheep resulted in a dose-dependent decrease of testis weight, simultaneous to a high and severe mortality rate (Honaramooz et al., 2005; Rasouli et al., 2020). Besides germ cells, the drug could also damage hematopoietic cells, contributing to significant systemic toxicity. It has caused drastic declines in the number of white blood cells and platelets (bone marrow toxicity) in sheep (Olejnik et al., 2018) and contributes to comparatively long-term testicular damage following injection in dogs (Hur et al., 2017).

On the contrary, mice have been reportedly resistant to it (Ganguli et al., 2016). Qin et al. (2016) demonstrated a slightly toxic effect with a testicular injection of busulfan, which surprisingly resulted in the success in SSC transplantation by restoring mice fertility and obtaining donor-derived offspring. This demonstrates that susceptibility to busulfan varies in species and is dose-dependent (Table 4). Meanwhile, testis irradiation, although shown to be more effective in depleting endogenous spermatogenesis than busulfan treatment in the domestic cat (*Felis catus*) (Silva et al., 2012), had resulted in permanent sterility in mice.

With large variations in susceptibility between organisms and negative effects of animals on busulfan and radiation therapy, recent studies are more focused on ex vivo gene editing of the NANOS2 gene using CRISPR/Cas9 to produce a genetically sterile male (Figure 6, TP: 9). To achieve this, researchers have created a male that lacks a functional copy of NANOS2, a highly conserved mammalian gene that plays an important role in the maintenance of SSC (Park et al., 2017). The inactivating mutations in the NANOS2 gene were developed using the CRISPR/Cas9 technology. Park et al. (2017) proved this by demonstrating that male pigs with bi-allelic frameshift mutations (knockouts) resulted in germline ablation but with seminiferous tubules that were morphologically unchanged. This approach has also been shown successfully in mice, pigs, goats, and cows (Ciccarelli et al., 2020). The author demonstrated that editing the NANOS2 gene by CRISPR/Cas9 renders males genetically sterile in different species and facilitates donor-derived spermatogenesis after allogeneic stem cell transplantation. There are no odd phenotypes other than a complete lack of endogenous SSC in the resulting animals

that are healthy. The transplantation of donor SSC into the otherwise physiologically normal testicular structure of these animals leads to the development of the donor cell SSC population and the production of donor sperm. The ultimate implementation of this technique would allow the transplantation of in vitro genetically modified cultured stem cells, creating functional gametes with altered (sterile) genotypes ready for SSC donor transplantation.

Table 4: Dosage of busulfan required for sterilization for various species.

Species	Optimum dose of busulfan (Required for sterilization)	References
Mice	5-45 mg/kg	Zohni et al., 2012
	30-44 mg/kg	Wang et al., 2010
Pigs	40-100 mg/kg	Kim et al., 1997
Coyotes	4-12 mg/kg	Stellflug et al., 1985
Monkey	8-12 mg/kg	Hermann et al., 2012
Dog	3.75 to 40 mg/kg	Deeg et al., 1999
	15-17.5 mg/kg	Hur et al., 2017

3.2.3 Spermatogonial stem cell transplantation

There are two types of SSC transplantation, allogeneic transplantation (same species) and xenogeneic transplantation (difference species; Figure 7). Allogeneic transplantation is an SSC transplant from a donor of the same species as the recipient. The SSC was isolated, expanded ex vivo, and transplanted in the same species of recipient male. The allogeneic transplantation performed in interspecies settings is called xenogeneic transplantation/xenotransplantation. Unlike allogeneic transplantation, which results in the successful production of spermatozoa, xenotransplantation has shown varied results (Table 5). The first xenotransplantation was performed in rodents by injecting rat donor cells into mouse testes and from mice to rats (Ogawa et al., 1999; Zhang et al., 2006). Both transplantations resulted in normal (morphologically and functionally) spermatozoa. However, complete spermatogenesis was obtained when performed on other combinations of rodents, e.g., between hamster to mouse, but most of the sperms exhibit abnormal characteristics (Ogawa et al., 1999). The extension of xenotransplantation approaches to other species (beside rodents) such as birds, dogs, horses, antelopes, and humans resulted in SSC colonization but did not progress beyond the spermatogonial phase (Dong et al., 2019; Ferrer et al., 2011; Goel et al., 2011; Mirzapour et al., 2015; Pereira et al., 2013; Pieri et al., 2019; Roe et al., 2013). This suggests that the phylogenetic divergence between recipients and donors can be a significant determinant of whether donor spermatogenesis would be supported by the recipient's environmental conditions. Recent xenotransplantation between closely related species proved this hypothesis. Silva et al. (2012) successfully reported colonization and differentiation of SSC to spermatozoa after 13 weeks of transplantation in felids, between ocelot (*Leopardus pardalis*) and domestic cat (*F. catus*). A similar result was also observed between the lion and domestic cat (Powell, 2015).

3.2.3.1 Application of SSCT in livestock breeding program

Generating infertile NANOS2 knockout males using gene editing allows new advanced reproductive breeding programs in livestock animals. Genetically sterile males could be used as surrogate sires, allowing for quicker transmission of certain characteristics like disease resistance, greater heat tolerance, or better meat quality. This technique is promising in speeding the distribution of favorable traits in cattle and improving the food supply for the world's expanding population. It would also provide breeders in remote areas easier access to elite animal genetic material from other parts of the world, allowing for more precise breeding in animals where artificial insemination (AI) is difficult.

Although AI has proven a powerful strategy for influencing genetic gain in livestock populations, its application has been limited to intensive production systems, and its impact relies on the ability to effectively cryopreserve sperm (Petruska et al., 2014). Unfortunately, these nuances are not conducive to the application of AI in extensive production systems, such as beef cattle operations, and with species for which sperm cryopreservation is limited, e.g., swine production. While AI is commonly employed in dairy cattle, which are frequently kept so that their reproductive behavior can be easily controlled, it is seldom utilized in beef cattle, which must roam freely to eat. Pig sperm does not withstand freezing well; thus, the technique still needs the presence of the animals (Roca et al., 2006). Moreover, because surrogates naturally transfer the donor genetic material through normal reproduction, the surrogate sire technology with SSC transplantation may overcome these

issues. Farmers may now let their animals interact naturally on the range or in the field. Donors and surrogates don't have to be in the same area because frozen donor sperm or the surrogate animal may be sent to other locations. This method has much promise for improving food security in developing countries, where farmers still have to rely on selective breeding to increase their herds.

Table 5: SSC transplantation in various species: (A) allogeneic transplantation and (B) xenogeneic transplantation.

	Donor	Recipient	Colonization	Spermatogenesis	Offspring	Reference
A	Chicken	Chicken	+	+	-	Yu et al., 2010
	Monkey	Monkey	+	+	-	Hermann et al., 2012
	Mice	Mice	+	+	+	Ganguli et al., 2016; Qin et al., 2016; Ciccarelli et al., 2020
	Goat	Goat	+	+	+	Ciccarelli et al., 2020
	Pig	Pig	+	+	+	Zeng et al., 2013; Ciccarelli et al., 2020
	Cattle	Cattle	+	+	+	Ciccarelli et al., 2020
B	Rat	Mice	+	+	-	Qu et al., 2012
	Mice	Rat	+	+	+	Ogawa et al., 1999
	Jundia catfish	Nile tilapia	+	+	-	Silva et al., 2016
	Japanese quails	Chickens	+	-	-	Pereira et al., 2012
	Dog	Mice	+	-	-	Pieri et al., 2019
	Horse	Rat	+	-	-	Ferrer et al., 2011
	Lion	Domestic cat	+	+	-	Powell, 2015
	Ocelot	Domestic cat	+	+	-	Silva et al., 2012
	Human (infant boy)	Mice	+	-	-	Dong et al., 2019
	Human	Mice	+	-	-	Mirzapour et al., 2014

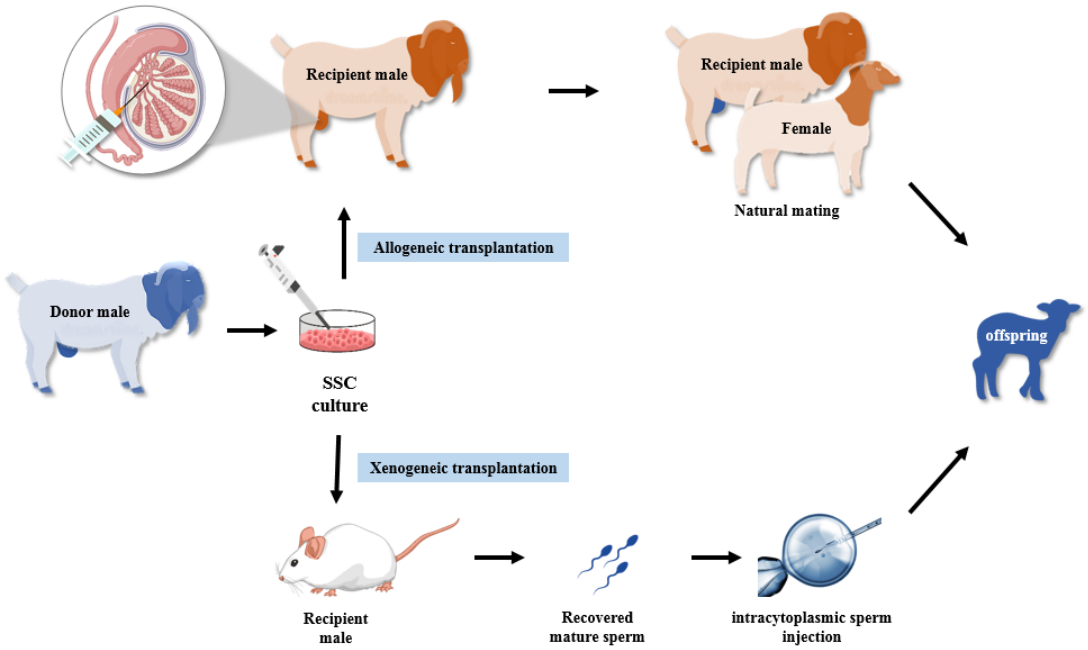


Figure 7: Types of SSC transplantation: allogeneic transplantation (same species) and xenogeneic transplantation (different species).

4. CONCLUSION

The analysis of 2931 publications over 59 years reveals that SSC research has significantly grown, especially in the last decade. Among these, 2171 (74%) are research articles, indicating a strong focus on empirical studies. SSC research predominantly focuses on three areas: SSC culture systems, techniques for generating ideal male recipients for transplantation, and SSC transplantation applications. The SSC culture system is the most researched topic, emphasizing the need for effective long-term culture systems. Establishing long-term SSC cultures for non-rodent species remains a challenge due to the complexity of replicating the in vivo niche environment. Techniques like using nanofiber scaffolds and optimizing culture conditions to favor glycolysis are being explored. Gene editing, particularly using CRISPR/Cas9 to create sterile males for SSC transplantation, shows promise in overcoming these challenges. Advances in SSC culture and transplantation can lead to new treatments for male infertility. By establishing long-term SSC cultures and improving transplantation techniques, SSC can be used to restore fertility in men with impaired spermatogenesis. Future research should focus on overcoming the challenges of long-term SSC culture, particularly for non-rodent species, and exploring the full potential of SSC applications in both medical and agricultural fields. The development of reliable SSC culture systems and efficient transplantation techniques will pave the way for groundbreaking advancements in reproductive medicine and livestock breeding.

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