Applications of Spermatogonial Stem Cell Technology in Wildlife Conservation

Sajida Mustafa[1,*], Hammad Hassan[1], Moattar Zahra[1], Sajid Abdullah[1], Mina Jamil[1] and Fatima Talib[1]

[1]Department of Zoology, Wildlife & Fisheries, University of Agriculture, Faisalabad, Pakistan
*Corresponding author: sajidamustafa59@yahoo.com

Abstract

Spermatogonial stem cells (SSCs) technology is the most popular and effective technique used in wildlife conservation to provide future strategies for conserving genetic variation and to rescue threatened species. SSCs, which are the primary sperm producing stem cells, can both, self-renew as well as differentiate into mature spermatozoa. The transplantation, cryopreservation, and it's in- vitro manipulation offer the chance to mitigate the effects resulting from habitat destruction, climate change and anthropogenic pressures. This particular chapter explore the various uses of SSC technology in the preservation of genetic resources, rehabilitation of species, interspecies germ cell transplantation, and developments in SSCs isolation, culture systems, and molecular characterization to improve utilization of these approaches. Moreover, philosophical and/or ethical factors and implications and/or possible adverse effects, for instance, the danger in genetic modification are outlined. Applying SSCs technology together with other biotechnological potentialities including somatic cell nuclear transfer, induced Pluripotent Stem cells could transform the prospects of ex-situ and in-situ conservation. Using SSCs technological applications, wildlife conservationists can protect biodiversity and help with sustainable ecosystem management.

Keywords: Stem Cell Technology, Wildlife Conservation, Genetic Modification, Ecosystem Protection

Cite this Article as: Mustafa S, Hassan H, Zahra M, Abdullah S, Jamil M and Talib F, 2025. Applications of spermatogonial stem cell technology in wildlife conservation. In: Nisa ZU, Altaf S, Zahra A and Saeed K (eds), Nanobiotech in Holistic Health: Innovations for Integrated Well-being. Unique Scientific Publishers, Faisalabad, Pakistan, pp: 85-90. https://doi.org/10.47278/book.HH/2025.105

A Publication of Unique Scientific Publishers	Chapter No: 25-013	Received: 12-Jan-2025 Revised: 25-March-2025 Accepted: 15-May-2025

Introduction

The constant reduction of numbers of animal species because of habitat loss, overexploitation and climate change has led to a need to create more effective technologies for conserving the animals (Hagedorn et al., 2018). For these difficulties Spermatogonial stem cells (SSCs) technology proved to be a breakthrough tool since it allows preserving genetic stock and performing species reclamations. SSCs are found in testes and play a crucial role in the ever replenishment process of sperm cells throughout the organism reproductive stage. Their capacity to self-multiply and to transform themselves into spermatozoa put them in a good position in wildlife conservation (Lara et al., 2018).

Wildlife in the world is under immense pressure as has never been witnessed before. Habitat degradation mainly due to communal grazing, conversion of land to agriculture, expansion of human settlement and wetland destruction has led to isolation of breeding sites and reduced availability of natural resource. At the same time, excessive killing by hunting and poaching and destructive fishing methods have pushed many different species to the edge of extinction (Guo et al., 2020). Even more, climate change fuels these difficulties worsening the conditions of ecosystems and making some kinds of habitats almost impossible. Such factors have the combined effect of causing a very fast rate of biome degradation, hence high extinction rates, which destabilize the ecosystems and their functions. Therefore, such calls for development of more effective strategies in the conservation and successful management of threatened species (Abofoul-Azab et al., 2019).

Of the diverse set of new biotechnological techniques, SSC technology has received considerable interest due to its possible application in conservation biology. SSCs are immature and morphologically non-specific gonocytes anchored in the seminiferous tubules of the testes (Irie et al., 2014). They are unlike most tissues in that they are bipotential for stem cell regeneration, thus providing an inexhaustible source of stem cells for the organism, and differentiation in order to generate mature spermatozoa. These characteristics make SSCs very important for male fertility and extremely useful for reproductive technologies (DeFalco et al., 2015). Current chapter explores the SSC technology in wildlife conservation and protection focusing on its potentials of genetic preservation, species restoration and reproductive biotechnology. Additionally, the advancements in SSC-based technologies are discussed. Furthermore, there are some ethical concerns and potential risks. Ultimately SSC discussed as valuable tool in biodiversity conservation and ecosystem as well as sustainable resource management.

The application of SSC technology in wildlife conservation can be broadly categorized into three domains.

Conservation of genetic resources under the techniques of isolation and culture of SSCs from pre-imaginal stages of endangered or threatened species (Ciccarelli et al., 2020). This method affords an opportunity to store SSCs in a manner that benefits the genetic variety of these species regardless of the fact that the natural population may reduce significantly to perilous levels. This approach augments ordinary gamete banking by offering possibility to check male germline cells that may be used to regain fertility and breeding in the future (Zhang et al., 2016).

In vitro fertilization has the potential to revitalize populations of many species with dwindling numbers. For example, SSC transplantation requires implantation of the donor SSCs directly into the testes of another male where it has the capacity to home in on the seminiferous tubules and differentiate to functional spermatozoa (Lord et al., 2022). This technique has been shown in several model organisms which includes mice and primates and has capabilities on interspecies. Hoping that SSCs of closely related surrogates could work for artificial insemination or in vitro fertilization, conservationists translocate SSCs from endangered species' testicular tissue into males belonging to the same species' group (Vazquez et al., 2016).

Specie conservation projects provides direction to genetic rescue of the species. This in fact means to revive the genetic variation in diminished small isolated subpopulations by trans-plant y genetic material preserved in SSCs (Hagedorn et al., 2018). Such interventions are necessary in order to prevent the consequences of inbreeding depression that worsen reproductive fitness and cause high levels of diseases. Through revitalizing the genetic variance, SSC-based approaches are indeed useful for increasing population recovery ability and overall adaptive capacity, both of which are important aspects of successful long-term population and species conservation (Jan et al., 2017).

Biology and Function of SSCS

SSCs are the undifferentiated male germ cells that sit in the base of the seminiferous tubule of the testis. These cells are important for male fertility because they provide the foundation for ongoing seminal tubing. SSCs up on initiation can only remain as stem cells through mitotic divisions, but at the same time actively produce progenitor cells that enter the pathway of spermatogenesis. This equilibrium, between self-renewal and differentiation, is maintained with a high precision so that the spermatogenic capacity is lifelong (Abofoul-Azab et al., 2019). The understanding of SSC regulation entails the use of both the intrinsic and extrinsic factors.

Intrinsic factors are main categories, which involve transcription factors that control self-renewal and differentiation of SSCs including PLZF, NANOS2, and FOXO1. These factors either activate or repress the genes which possess importance to retain stem cell identity or to start the differentiation program (Jan et al., 2017).

Extrinsic factors play the same role and are also important, SSC niche offers conduits that determine their destiny. Special groupings include Sertoli cells, peritubular myoid cells or any other supporting cell found in the seminiferous tubules (Hermann et al., 2018). Chemical factors expressed by the niche encompass GDNF, FGF, and CSF1 among others for the neuronal, growth factor and cytokine families respectively. Sertoli cells synthesize GDNF, which is critical for SSC (utilized throughout the textual passage) self-renewal and FGF and CSF1 are involved in SSC proliferation and survival. Moreover, basal lamina and collagen present nearby Sertoli cells with structural support as well as biochemical signals that help them to be anchored and signalized (Costa et al., 2012).

Thus, constant reciprocal interplay between the intrinsic cell properties and the extrinsic signals guarantees the homeostasis of SSCs and the ability of adult males to produce several millions of sperm per day. Aberrations in these mechanisms may result in infertility or other related pathologies meaning that advances in SSC biology were necessary for the development of related therapies (Figure 1) (Martinez et al., 2020).

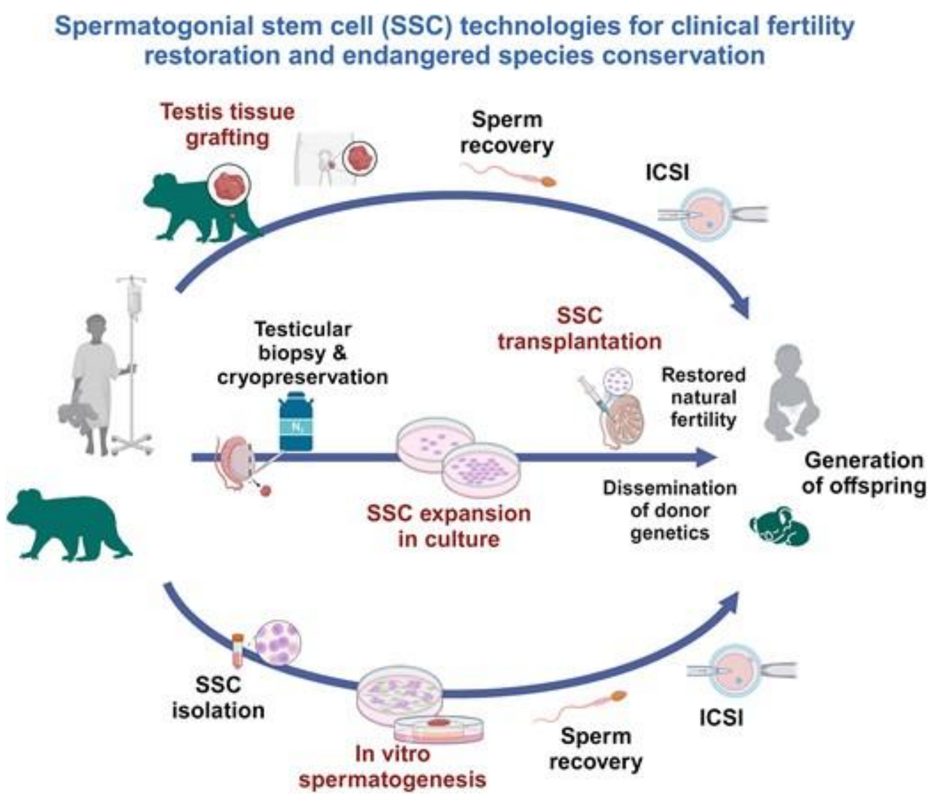

Fig. 1: SSC technology for treating infertility in endangered species (Damyanova et al., 2024)

Applications of SSC Technology in Wildlife Conservation

Genetic Resource Banking

SSCs based genetic resource banking is a revolutionary concept in conservation biology that can potentially provide unique solutions for effective conservation of genetic stocks of endangered species. Despite the fact while SSC cryopreservation is distinct from sperm banking, through which sperms are predesigned for make use of at any later date, but there are possibility of creating sperms in an unlimited fashion given proper in vitro or in vivo conditions (Mall et al., 2020). This feature is especially beneficial for the species that have specific problems connected with their reproduction, for example, these having the seasonal character in their reproduce duties and in which the sperm supply is present only during the certain periods of the year. Furthermore, SSC banking can eliminate problems concerning the cryopreservation of mature sperm since in some species their sperm is easily damaged; or in other cases, the effectiveness of the sperm is low (Devi et al., 2014).

A distinct strength to SSC banking is that it is suitable for prepubertal people. When young males of endangered species die before reproducing, the remains with their SSCs collected and preserved, useful for future generations or populations. Likewise, SSCs can be obtained at postmortem of individuals depending on the tissue samples collected and preserved shortly after the death. This capability greatly extends the original concept of genetic resource banking because it provides a means of genetic material storage even in emergent situations (Andrae et al., 2021).

Moreover, SSC cryopreservation facilitates the creation of biobanks, where only all sorts of genetic materials of various individuals, within a particular species, can be stored. Such biobanks proved to be useful for researches and conservation, as they can act as the source of genetic material for the following experiments or the breeding stock for the species (Binsila et al., 2018). Recent improvements in vitrification or other methods of mammalian gamete freezing have given increased post-thaw survival and function to SSCs, thus consolidating this technology for conservation purposes (Zehmal et al., 2020).

Assisted Reproductive Technologies (ARTs)

As for SSC technology, although it has not been widely used in ARTs, its incorporation has broadened the enlarged the application and effectiveness of ARTs in wildlife conservation. Ectopic transplantation of SSCs into recipient testes is a novel procedure that allows for the generation of sperm from donor derived SSCs. This approach allows those with low fertility or skewed sex ratios to pass on their genes, solving two major biological problems in endangered species (Oatley et al., 2016).

Owing to the inability to perform some traditional ARTs such as artificial insemination or in vitro fertilization because sperm is lacking or difficult to obtain in good quality, SSC transplantation is most useful for species that require assisted reproduction techniques. Therefore, by transplanting SSCs into the testes of a recipient male sperm can be produced naturally without a need for repeated interventions. This technique has been successfully used in a number of mammal models and this work sets the stage for using these techniques for improved conservation of wildlife (Mall et al., 2020).

Another advancement in the arena of SSC research is the cross-species SSC transplantation. In this case, SSCs from a donor species are transplanted in the testes of a phylogenetically close related species which then serves as surrogate for spermatogenesis (Johnston, 2019). This strategy has been advantageous for those poorly represented species which closely related species are common but easily accessible. For example, SSCs have been taken from endangered felids and then transplanted into surrogate domestic cats, in which viable sperm was developed. Such cross-application also offers heightened probabilities of improving the reproductive performance of threatened species, besides easing the invasive ways of collection (Pothana et al., 2016).

However, ARTs that embraces SSC technology can solve reproductive difficulties in such species with many social structure or behavioral restrictions. For instance, in a species where dominant male genitors control all the breeding opportunities then SSC transplantation can increase the genetic representation of subordinate genitors and improve genetic subpopulation within founding stock (Yang et al., 2018).

Species Restoration

SSC technology appliqué in species restoration and what can be deemed as the de-extinction is living evidence of this reality. Through utilizing preserved SSCs, lost heritable characters may be brought into breeding populations or even create new species other such advanced means of breeding are employed. This approach provides a special chance to solve the problems of genetic erosion and population depletion which endanger the variety of life on the earth (Devi et al., 2014).

Among all the promising uses of SSC technology in species restoration, the use of surrogate species has been of significant importance. In the method, the preserved SSCs from an endangered or an extinct species are then implanted in to the testes of a closely related species (Sahare et al., 2014). Thereafter, the surrogate also provides sperm that contains genetic characteristics of the donor species to be used in fertilization and breeding. This technique has been implemented in several species, for example, felids, SSCs from endangered cats have been put in domestic cats surrogate where they gave birth alive and healthy offspring (Lee et al., 2014).

The application of SSC technology in de-extinction has attracted much attention according to various techniques in reestablishing the species that were eradicated by various human interventions (Paul et al., 2017).The successful transplantation of SSCs' genetic material into current populations provides the opportunity to restore lost genes that provided specimens with durability and flexibility they otherwise would not possess helping species overall survival against their diminishing environments. For example, there is intentionally breeds some SSCs from the former population to the present population of an endangered species, thus following the loss of genetic variation and inbreeding depression (Kim et al., 2015). The survival and successful reintroduction of SSC-based species is therefore a function of improvements in cryopreservation, transplantation, and in-vitro culture. Present studies focus on fine-tuning of these techniques to enhance the immediate and durable functionality of SSCs after transplantation. Moreover, concerns on the ethic of the project and relevance to the overall environmental objectives and impact to the ecology of the area must also be given thorough consideration to confirm that the results of the species rehabilitation are in with modern conservation vision (Kong et al., 2018).

Genetic Diversity Enhancement

Genetic management is one of the most fundamental concerns for wildlife conservation and SSC technology is developed to serve that purpose. Although, it is good practice to be extra cautious when transplanting animals and plants from one place to another, genetic variability plays an important role in the survival and the wellbeing of endangered populations because species need it in order to counter changes in the environment; to fend off diseases; and to improve the general health of the population without which, many species are likely to go extinct. SSCs are useful tools for bringing new alleles into a population and reducing such problems of small isolated populations as inbreeding and genetic drifts (Patra et al., 2021).

In many endangered species, loss of genetic variation due to small population size, gene flow and habitat fragmentation, increases the accumulation of bad genes. The fundamentals of SSCs can also help solve such obstacles to the extent that SSCs can act as a way of transferring genetic material derived from genetically differentiated individual or populations. Through grafting of SSCs into recipient males, sperms harboring different gene characteristics can be generated for artificial insemination or natural mating. It may take a positive turn and perhaps counter the ill effects of inbreeding and increase the general well-being of endangered species (Suyatno et al., 2018).

SSC application for genetic improvement is most appropriate for species whose breeding patterns are not so straightforward or do not have knowable social systems. In species where dominant males control mating, subordinate males will rarely or never assist in gene pool creation. Through the SSC transplantation, the genetic material of these individuals is well represented hence equality in distribution of the same amongst the population (Pramod et al., 2023).

Advances in SSC Technology

- Some changes have though been achieved on the scope of cryopreservation in SSCs such as vitrification and use of cryoprotectant. Such techniques guarantee that the genetic material stays viable and will be helpful for further uses in preservation (Kim et al., 2015).
- The SSCs has also been greatly witnessed by RNA-seq and ChIP-seq Next-generation sequencing technologies to unravel and better understand the SSC transcriptome and epigenome that has in turn informed culture and transplantation strategies (Yang et al., 2018).
- Attempts to reproduce spermatogenesis have yielded positive results in mice through production of testicular elongated cells from SSCs. This could eliminate the need for the use of animals and their characteristics would not be reproduced through transplantation between species as it has been a huge concern (Suyatno et al., 2018).

Ethical and Ecological Concerns

Ethical and ecological concerns are important for those of us who are living now they constitute the germination of tough questions that should be posed to society and result from understanding the importance of human life and the environment (Shah et al., 2018). SSC technology can make a significant difference, yet, their application is questionable both from ethical and ecological aspect. Genetic engineering could also bring insertional mutagenesis, or lead to population disruption. Furthermore, the case with the selection of target species for SSC based interventions may distort other conservation objectives in the ecosystem. Prominent decision making patterns should be transparent along with tough risk analysis to combat these potential issues (Lee et al., 2014).

Future Directions

1. Relation with Other Biotechnologies

The use of SSC technology integrated with somatic cell nuclear transfer (SCNT), jointly with gene editing tools such as CRISPR-Cas9, could improve significance and range of its application. For example, one may modify genes in SSCs to get rid of detrimental changes that can harm the animal before their utilization in conservation (Kong et al., 2018).

2. Towards Wider Cross Species Compatibility

Studies exploring how to increase the cross species SSC transplantation success may expand the list of suitable surrogates making this technology more useful in preserving the critically endangered species (Pramod et al., 2023).

3. Participation and Policy-making

Mainly due to the distinct potential impacts of the SSC technology in conservation, there is a strong call for public awareness and stakeholder engagement in the ethical application of the technology. It will thereby be possible to adopt policies that respond to technological progress while at the same time preserving the ecological and cultural environments (Mall et al., 2020).

Conclusion

SSC technology has great potential to counterpart continuing biodiversity threat like conservation of genetic assets, recovery of endangered species and improvement of genetic stock. Being part of the conservation sector, integration of those emerging biotechnologies like gene editing, reproducing spermatogensis and cryopreservation together with strict compliance to ethical issues will be the decisive factors for its success in wildlife conservation. Utilizing these viewpoints that were developed through the improved SSC-based interventions, we can establish a less volatile environment in the existence of world’s wildlife and the ecosystems they call home, in essence the strengthen the stability of our earth’s biodiversity.

References

Abofoul-Azab, M., Lunenfeld, E., Levitas, E., Zeadna, A., Younis, J., Bar-Ami, S., & Huleihel, M. (2019). Identification of Premeiotic, Meiotic, and Postmeiotic Cells in Testicular Biopsies Without Sperm from Sertoli Cell-Only Syndrome Patients. *International Journal of Molecular Sciences*, *20*(3), 470. https://doi.org/10.3390/ijms20030470

Andrae, C. S., Oliveira, E. C., Ferraz, M. A., & Nagashima, J. B. (2021). Cryopreservation of grey wolf (Canis lupus) testicular tissue. *Cryobiology*, *100*, 173–179. https://doi.org/10.1016/j.cryobiol.2021.01.010

Binsila, K. B., Selvaraju, S., Ghosh, S. K., Parthipan, S., Archana, S. S., Arangasamy, A., Prasad, J. K., Bhatta, R., & Ravindra, J. P. (2018). Isolation and enrichment of putative spermatogonial stem cells from ram (Ovis aries) testis. *Animal Reproduction Science*, *196*, 9–18. https://doi.org/10.1016/j.anireprosci.2018.04.070

Ciccarelli, M., Giassetti, M. I., Miao, D., Oatley, M. J., Robbins, C., Lopez-Biladeau, B., Waqas, M. S., Tibary, A., Whitelaw, B., Lillico, S., Park, C., Park, K., Telugu, B., Fan, Z., Liu, Y., Regouski, M., Polejaeva, I. A., & Oatley, J. M. (2020). Donor-derived spermatogenesis following stem cell transplantation in sterile NANOS2 knockout males. *Proceedings of the National Academy of Sciences*, *117*(39), 24195–24204. https://doi.org/10.1073/pnas.2010102117

Costa, G. M. J., Avelar, G. F., Rezende-Neto, J. V., Campos-Junior, P. H. A., Lacerda, S. M. S. N., Andrade, B. S. C., Thomé, R. G., Hofmann, M., & Franca, L. R. (2012). Spermatogonial stem cell markers and niche in equids. *PLoS ONE*, *7*(8), e44091. https://doi.org/10.1371/journal.pone.0044091

Damyanova, K. B., Nixon, B., Johnston, S. D., Gambini, A., Benitez, P. P., & Lord, T. (2024). Spermatogonial stem cell technologies: applications from human medicine to wildlife conservation. *Biology of Reproduction*, *111*(4), 757–779. https://doi.org/10.1093/biolre/ioae109

De Lima E Martins Lara, N., Costa, G. M. J., Avelar, G. F., Guimarães, D. A., & França, L. R. (2018). Postnatal testis development in the collared peccary (Tayassu tajacu), with emphasis on spermatogonial stem cells markers and niche. *General and Comparative Endocrinology*, *273*, 98–107. https://doi.org/10.1016/j.ygcen.2018.05.013

DeFalco, T., Potter, S. J., Williams, A. V., Waller, B., Kan, M. J., & Capel, B. (2015). Macrophages contribute to the spermatogonial niche in the adult testis. *Cell Reports*, *12*(7), 1107–1119. https://doi.org/10.1016/j.celrep.2015.07.015

Devi, L., Makala, H., Pothana, L., Nirmalkar, K., & Goel, S. (2014). Comparative efficacies of six different media for cryopreservation of immature buffalo (Bubalus bubalis) calf testis. *Reproduction Fertility and Development*, *28*(7), 872. https://doi.org/10.1071/rd14171

Guo, J., Nie, X., Giebler, M., Mlcochova, H., Wang, Y., Grow, E. J., Kim, R., Tharmalingam, M., Matilionyte, G., Lindskog, C., Carrell, D. T., Mitchell, R. T., Goriely, A., Hotaling, J. M., & Cairns, B. R. (2020). The Dynamic Transcriptional Cell Atlas of Testis Development during Human Puberty. *Cell Stem Cell*, *26*(2), 262-276.e4. https://doi.org/10.1016/j.stem.2019.12.005

Hagedorn, M. M., Daly, J. P., Carter, V. L., Cole, K. S., Jaafar, Z., Lager, C. V. A., & Parenti, L. R. (2018). Cryopreservation of fish spermatogonial cells: the future of Natural History collections. *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-24269-3

Hermann, B. P., Cheng, K., Singh, A., La Cruz, L. R., Mutoji, K. N., Chen, I., Gildersleeve, H., Lehle, J. D., Mayo, M., Westernströer, B., Law, N. C., Oatley, M. J., Velte, E. K., Niedenberger, B. A., Fritze, D., Silber, S., Geyer, C. B., Oatley, J. M., & McCarrey, J. R. (2018). The Mammalian Spermatogenesis Single-Cell Transcriptome, from Spermatogonial Stem Cells to Spermatids. *Cell Reports*, *25*(6), 1650-1667.e8. https://doi.org/10.1016/j.celrep.2018.10.026

Irie, N., Tang, W. W. C., & Surani, M. A. (2014). Germ cell specification and pluripotency in mammals: a perspective from early embryogenesis. *Reproductive Medicine and Biology*, *13*(4), 203–215. https://doi.org/10.1007/s12522-014-0184-2

Jan, S. Z., Vormer, T. L., Jongejan, A., Röling, M. D., Silber, S. J., De Rooij, D. G., Hamer, G., Repping, S., & Van Pelt, A. M. M. (2017). Unraveling transcriptome dynamics in human spermatogenesis. *Development*, *144*(20), 3659–3673. https://doi.org/10.1242/dev.152413

Johnston, S. (2019). Challenges associated with the development and transfer of assisted breeding technology in marsupials and monotremes: lessons from the koala, wombat and short-beaked echidna. *Reproduction Fertility and Development*, *31*(7), 1305. https://doi.org/10.1071/rd18113

Kim, K., Lee, Y., Kim, B., Kim, Y., Kim, B., Kang, H., Jung, S., Choi, S., Schmidt, J. A., & Ryu, B. (2015). Cryopreservation of putative pre-pubertal bovine spermatogonial stem cells by slow freezing. *Cryobiology*, *70*(2), 175–183. https://doi.org/10.1016/j.cryobiol.2015.02.007

Kong, L., Qiu, L., Guo, Q., Chen, Y., Zhang, X., Chen, B., Zhang, Y., & Chang, G. (2018). Long-term in vitro culture and preliminary establishment of chicken primordial germ cell lines. *PLoS ONE*, *13*(4), e0196459. https://doi.org/10.1371/journal.pone.0196459

Lee, Y., Kim, Y., Ha, S., Kim, K., Kim, B., Kim, B., Choi, S., Kim, I., Schmidt, J. A., & Ryu, B. (2014). Cryopreservation of porcine spermatogonial stem cells by slow-freezing testis tissue in trehalose1. *Journal of Animal Science*, *92*(3), 984–995. https://doi.org/10.2527/jas.2013-6843

Lord, T., Law, N. C., Oatley, M. J., Miao, D., Du, G., & Oatley, J. M. (2022). A novel high throughput screen to identify candidate molecular networks that regulate spermatogenic stem cell functions. *Biology of Reproduction*, *106*(6), 1175–1190. https://doi.org/10.1093/biolre/ioac048

Mall, E. M., Rotte, N., Yoon, J., Sandhowe-Klaverkamp, R., Röpke, A., Wistuba, J., Hübner, K., Schöler, H. R., & Schlatt, S. (2020). A novel xeno-organoid approach: exploring the crosstalk between human iPSC-derived PGC-like and rat testicular cells. *Molecular Human Reproduction*, *26*(12), 879–893. https://doi.org/10.1093/molehr/gaaa067

Martinez, M. P., Elbardisi, H., Majzoub, A., & Arafa, M. (2020). Epidemiology of Genetic Disorders in male Infertility. In *Springer eBooks* (pp. 73–94). https://doi.org/10.1007/978-3-030-37972-8_5

Oatley, M. J., Kaucher, A. V., Yang, Q., Waqas, M. S., & Oatley, J. M. (2016). Conditions for Long-Term Culture of cattle undifferentiated spermatogonia. *Biology of Reproduction*, *95*(1), 14. https://doi.org/10.1095/biolreprod.116.139832

Patra, T., Pathak, D., & Gupta, M. K. (2021). Comparison of two culture methods during in vitro spermatogenesis of vitrified-warmed testis tissue: Organ culture vs. hanging drop culture. *Cryobiology*, *100*, 142–150. https://doi.org/10.1016/j.cryobiol.2021.02.006

Paul, R., Bahire, S., & Kumar, D. (2017). Isolation and biochemical characterization of ovine spermatogonial stem cells. *Indian Journal of Small Ruminants (The)*, *23*(2), 186. https://doi.org/10.5958/0973-9718.2017.00041.1

Pothana, L., Devi, L., & Goel, S. (2016). Cryopreservation of adult cervid testes. *Cryobiology*, *74*, 103–109. https://doi.org/10.1016/j.cryobiol.2016.11.008

Pramod, R. K., Varughese, D., Jameel, A. J., Panda, B. N., Goswami, S., & Mitra, A. (2023). Cryopreserved ovine spermatogonial stem cells maintain stemness and colony forming ability in vitro. *Asian Pacific Journal of Reproduction*, *12*(6), 273–280. https://doi.org/10.4103/2305-0500.390302

Sahare, M., Otomo, A., Komatsu, K., Minami, N., Yamada, M., & Imai, H. (2014). The role of signaling pathways on proliferation and self-renewal of cultured bovine primitive germ cells. *Reproductive Medicine and Biology*, *14*(1), 17–25. https://doi.org/10.1007/s12522-014-0189-x

Shah, M. A., Xu, C., Wu, S., Zhao, W., Luo, H., Yi, C., Liu, W., & Cai, X. (2018). Isolation and characterization of spermatogenic cells from cattle, yak and cattleyak. *Animal Reproduction Science*, *193*, 182–190. https://doi.org/10.1016/j.anireprosci.2018.04.067

Suyatno, N., Kitamura, Y., Ikeda, S., Minami, N., Yamada, M., & Imai, H. (2018). Long-term culture of undifferentiated spermatogonia isolated from immature and adult bovine testes. *Molecular Reproduction and Development*, *85*(3), 236–249. https://doi.org/10.1002/mrd.22958

Vazquez, B. N., Thackray, J. K., Simonet, N. G., Kane-Goldsmith, N., Martinez-Redondo, P., Nguyen, T., Bunting, S., Vaquero, A., Tischfield, J. A., & Serrano, L. (2016). SIRT 7 promotes genome integrity and modulates non-homologous end joining DNA repair. *The EMBO Journal*, *35*(14), 1488–1503. https://doi.org/10.15252/embj.201593499

Yang, C., Yao, C., Tian, R., Zhu, Z., Zhao, L., Li, P., Chen, H., Huang, Y., Zhi, E., Gong, Y., Xue, Y., Wang, H., Yuan, Q., He, Z., & Li, Z. (2018). MIR-202-3P regulates sertoli cell proliferation, synthesis function, and apoptosis by targeting LRP6 and cyclin D1 of WNT/B-Catenin signaling. *Molecular Therapy — Nucleic Acids*, *14*, 1–19. https://doi.org/10.1016/j.omtn.2018.10.012

Zahmel, J., Jänsch, S., Jewgenow, K., Sandgreen, D., Simonsen, K. S., & Colombo, M. (2020). Maturation and fertilization of African lion (Panthera leo) oocytes after vitrification. *Cryobiology*, *98*, 146–151. https://doi.org/10.1016/j.cryobiol.2020.11.011

Zhang, T., Oatley, J., Bardwell, V. J., & Zarkower, D. (2016). DMRT1 is required for mouse spermatogonial stem cell maintenance and replenishment. *PLoS Genetics*, *12*(9), e1006293. https://doi.org/10.1371/journal.pgen.1006293