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GENERATION OF SPERMATOGONIA FROM HUMAN AND NON-HUMAN PRIMATE PLURIPOTENT STEM CELLS: CURRENT PROGRESS, CHALLENGES AND FUTURE PERSPECTIVES

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Abstract:

Treating male infertility caused by the absence of germ cells remains a major biomedical challenge. To address this, scientists are investigating cell therapy utilizing pluripotent stem cells differentiated into spermatogonia. While animal models, particularly mice, have provided vital insights into primate and human reproductive biology, replicating this differentiation process under human-like culture conditions remains prolonged and complex. Researchers currently face substantial hurdles, including effectively separating target spermatogonia from other cellular fractions. Although lab-generated cells can mimic the phenotypic and molecular traits of spermatogonia, producing fully functional human spermatogonial stem cells capable of sustaining ongoing spermatogenesis remains unachieved. Consequently, clinical transplantation is not viable in the foreseeable future. Key obstacles must be overcome before human trials can proceed. Researchers must generate the vast cell quantities required for therapy while resolving critical safety questions. Notably, the differentiation process carries oncological risks due to potential genetic mutations, necessitating protocols that ensure defect-free cells. Comprehensive safety and feasibility data from animal models must be established first. Ultimately, clinical trials will only commence once scientists develop a reliable, mutation-free method to guide stem cells into functional germ cells..

Keywords: *Spermatogonia, Pluripotent Stem Cells, In-vitro Spermatogenesis, Non-Human Primates, Male Infertility.*

1. Introduction

Failures in germline development cause many cases of male infertility. For patients who cannot produce sperm, treatments like intracytoplasmic sperm injection are often impossible because they require at least some viable testicular sperm [1]. Researchers are trying to solve this by creating functional male germ cells in the laboratory [2].

To do this, they often start with pluripotent stem cells (PSCs). These cells can differentiate into almost any tissue type. By applying specific growth factors, scientists can guide PSCs through the early stages of germ cell development [3, 4]. They first become primordial germ cell-like cells, which then mature into spermatogonia [5, 6]. Spermatogonia act as the stem cells of the testes. They divide to maintain their own numbers while also producing the cells that eventually become mature sperm [7].

Most early experiments used mice. Mouse embryonic stem cells successfully completed the entire male germ cell development cycle *In-vitro* , producing functional sperm that led to healthy offspring [8, 9]. However, rodent spermatogenesis differs in several ways from primate spermatogenesis [5, 10]. To develop safe treatments for humans, researchers need models that closely mirror human biology. This makes non-human primates, like rhesus macaques, highly useful for testing these procedures [11, 12].

This article reviews how scientists generate spermatogonia from human and non-human primate PSCs. It looks at the methods used, the markers that identify successful differentiation, and the challenges that remain before these techniques can help patients.

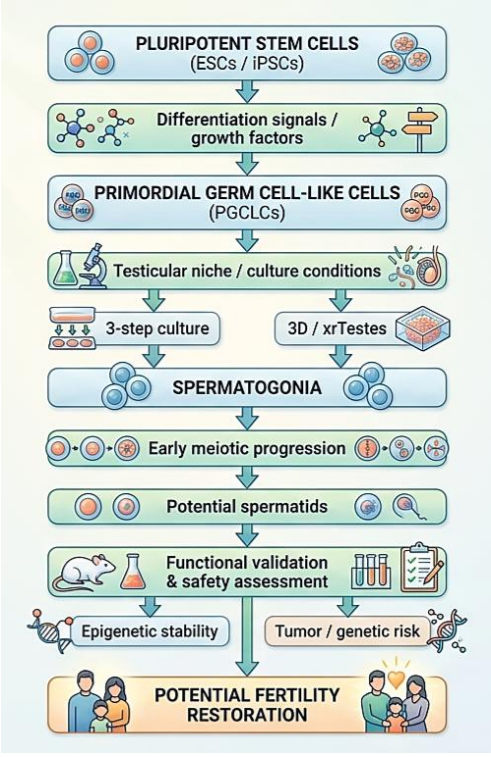

Figure 1: *In-vitro* Differentiation Pathway for the Generation of Spermatogonia from Pluripotent Stem Cells

2. Differentiation strategies in humans and non-human primates

Creating spermatogonia from PSCs requires mimicking the natural environment of the developing testes. The first step involves inducing PSCs to form primordial germ cell-like cells [5].

Once these early cells form, they need a specific physical and chemical environment to progress further. In vivo, the testicular microenvironment, or niche, provides the necessary signals [13, 14]. To recreate this outside the body, some protocols use a three-step culture system. This approach forces the cells through specific

developmental stages until they express markers associated with spermatogonial stem cells [15]. These *In-vitro* -derived cells can sometimes propagate for months and maintain their morphological properties [15].

Another method uses three-dimensional structures. Some research teams mix human or primate germ cell-like cells with mouse fetal testicular somatic cells [5]. These cells aggregate and organize into structures resembling seminiferous tubules, known as xenogeneic reconstituted testes (xrTestes). When transplanted into immunodeficient mice, the xrTestes support the maturation of the human or rhesus macaque cells into spermatogonia [5]. These cells can even reach the preleptotene stage of meiosis, showing that the physical structure of the tubule helps drive development [5]. Recent studies have even derived human induced pluripotent stem cells (iPSCs) from cryopreserved testicular tissue of childhood cancer survivors [6]. These iPSCs successfully differentiated back into primordial germ cell-like cells. This suggests a potential pathway for patients whose germ cell pools were depleted by chemotherapy [6, 16].

3. Differentiation strategies for generating spermatogonia

Table 1: Differentiation strategies for generating spermatogonia

Approach	Starting Material	Main Strategy	Outcome	Major Limitation
Three-step culture system	Human/primate PSCs	Sequential differentiation through germ-cell developmental stages	Cells expressing markers associated with spermatogonial stem cells	Long and technically demanding process
3D / xrTestes system	Human or macaque germ-cell-like cells	Co-culture with mouse fetal testicular somatic cells to form seminiferous-tubule-like structures	Differentiation into spermatogonia and progression toward early meiosis	Requires xenogeneic support and transplantation into immunodeficient mice
Direct PSC differentiation	Human ESCs/iPSCs	Use of specific growth factors to induce germ-cell development	Generation of germ-cell/spermatogenic cells	Functional maturity remains difficult to establish
Patient-derived iPSC approach	iPSCs from cryopreserved testicular tissue	Reprogramming followed by germ-cell differentiation	Generation of primordial germ cell-like cells	Still an early experimental approach
Mouse PSC model	Mouse embryonic stem cells	In-vitro reconstruction of male germ-cell development	Complete spermatogenesis and functional sperm reported	Mouse biology does not completely reproduce human/primate spermatogenesis
Primate PSC model	Macaque iPSCs/ESCs	Differentiation using testicular microenvironment/ xrTestes	Spermatogonia and early meiotic cells; spermatid-like cells reported	Technically demanding and dependent on xenogeneic systems

4. Identifying reliable markers

Validating the identity of *In-vitro* -derived cells is difficult. Because researchers cannot easily test human cells for their ability to produce living offspring, they rely on molecular markers to confirm that a cell is actually a

spermatogonium. This reliance on markers sometimes leads to errors. Early studies attempting to culture spermatogonia from primate testes actually produced testicular multipotent stromal cells instead [17]. These stromal cells express many of the same surface markers commonly used to identify spermatogonia, such as GPR125, CD90, and ITGA6 [17]. To avoid this confusion, researchers must use a combination of markers. VASA, when combined with MAGEA4, PLZF, and SALL4, provides a more accurate way to distinguish true spermatogonia from multipotent stromal cells [17]. Accurate identification ensures that experiments measure actual germ cell progress rather than the growth of supportive tissue.

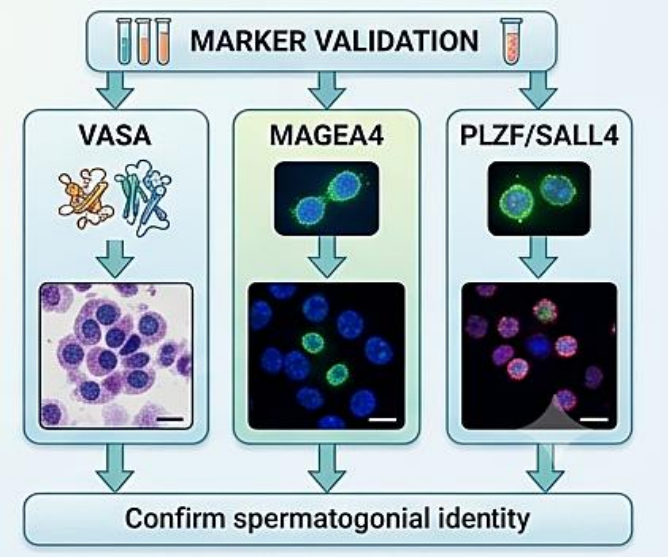

Figure 2: Molecular Marker-Based Validation of *In-vitro* -Derived Spermatogonia

Table 2: Markers used to identify spermatogonia

Marker	Role in identification	Interpretation
VASA	Germ-cell marker	Supports germ-cell identity
MAGEA4	Spermatogonial/germ-cell marker	Helps distinguish spermatogonia
PLZF	Spermatogonial stem/progenitor marker	Supports spermatogonial identity
SALL4	Germ-cell developmental marker	Supports germ-cell identity
GPR125	Surface marker	Can also be expressed by stromal cells
CD90	Surface marker	Not specific enough alone
ITGA6	Surface marker	Can overlap with multipotent stromal cells

5. Comparative *In-vitro a*pproaches

Efforts to derive male germ cells from pluripotent stem cells exhibit distinct, species-specific outcomes. Mouse models demonstrate unprecedented success, achieving complete in-vitro spermatogenesis and generating viable progeny. In contrast, primate and human systems encounter major translational bottlenecks. Although human and macaque models yield early meiotic precursors or spermatid-like cells, clinical application remains constrained by a heavy reliance on xenogeneic support, demanding culture protocols, and ethical barriers that preclude definitive in-vivo reproductive validation.

Table 3: Comparative *In-vitro A*pproaches

Model System	Source Material	Key Outcomes	Limitations
Mouse Models	Embryonic stem cells	Complete *In-vitro* spermatogenesis; fertile offspring produced [8, 9].	Biological differences make direct translation to humans unreliable [5].
Non-Human Primates (Macaque)	iPSCs	Differentiation into spermatogonia and early meiotic cells within xrTestes [5]. Generation of functional spermatid-like cells [19].	Protocols are technically demanding and require xenogeneic support [11].
Human Models	ESCs and patient-derived iPSCs	Generation of haploid spermatogenic cells [20] and propagation of spermatogonial stem cells [15]. Reconstitution of epigenetic reprogramming [21].	Cannot functionally test in vivo through reproduction; relies heavily on marker validation [17].

6. Challenges and future perspectives

Producing male gametes in the laboratory faces technical and safety barriers. Achieving fully functional human spermatozoa *In-vitro* remains difficult. Most current systems stall before completing meiosis or produce cells with abnormal shapes [16]. Safety is the main concern for clinical application. Stem cells inherently carry a risk of forming tumors if they remain undifferentiated upon transplantation [16]. Reintroducing cells into a patient requires absolute certainty that no malignant cells remain in the graft. Epigenetic programming also presents a challenge. Male germ cells undergo extensive DNA methylation changes during their development [21]. If *In-vitro* systems fail to replicate this exact sequence, the resulting cells could carry epigenetic defects that might cause developmental disorders [22].

7. Conclusion

Generating spermatogonia from pluripotent stem cells offers a possible treatment for severe male infertility. Researchers have successfully guided human and macaque stem cells through the early stages of germline development. Co-culturing these cells with testicular somatic cells helps them organize into tubule-like structures and progress toward meiosis. While non-human primate models improve the reliability of these studies, translating the technology to human medicine requires solving problems related to epigenetic stability and tumor risks.

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