



Reconstructing The Testicular Niche: Mechanisms and Translational Perspectives of Stem Cell-derived Testicular Organoids*

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Abstract Mammalian male germline development depends on a specialized gonadal somatic microenvironment. However, its *in vitro* reconstruction remains challenging. Major barriers include the generation of appropriate supporting-cell lineages, organized tissue architecture, and culture conditions that sustain germ-cell maturation. Yoshino *et al.* established a mouse pluripotent stem cell-derived testicular organoid model. This model reconstitutes the sex-determining region Y (Sry) -SRY-box transcription factor 9 (Sox9)-mediated sex-determination program and generates testicular somatic cell-like cells (TesLCs). When assembled with primordial germ cell-like cells (PGCLCs), TesLCs formed seminiferous tubule-like structures and supported male germline progression toward prospermatogonia and germline stem cell-like cells (GSCLCs). After transplantation into infertile recipient testes, organoid-derived GSCLCs produced fertilization-competent sperm and generated fertile offspring. The major significance of this work extends beyond sperm production. It integrates sex determination, germ-cell fate transition, and testicular niche self-organization into an experimentally tractable organoid system. This commentary discusses the technical strategy, sex-determination mechanism, value of testicular niche reconstruction, and translational limitations of this study. It also highlights its implications for *in vitro* gametogenesis, male infertility modeling, and reproductive medicine.

Key words pluripotent stem cells, sex determination, testicular organoid, *in vitro* gametogenesis, reproductive microenvironment

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A major challenge in *in vitro* gametogenesis is no longer only to obtain germ cells. It is also to reconstruct the reproductive niche. Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) can be induced into primordial germ cell-like cells (PGCLCs). However, their further maturation often requires embryonic gonads or an *in vivo* transplantation environment. This suggests that germ-cell fate is not determined by germ cells alone. It is also shaped by gonadal somatic cells, the basement membrane, interstitial cells, and three-dimensional tissue architecture. Yoshino *et al.*^[1] started from mouse pluripotent stem cells (PSC). They sequentially reconstructed a bipotential gonadal state, sex determination, testicular somatic-cell lineages, testicular organoids, and male germline progression. Their study enables *in vitro* tracking and perturbation

of microenvironment-driven sex determination.

1 Key advance: from pluripotent stem cells to a testicular organoid model

The study used a stepwise developmental strategy. First, the authors generated Sox9-green fluorescent protein (Sox9-GFP), Foxl2-tdTomato, and Nr5a1-human CD271 (Nr5a1-hCD271) reporter

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systems. These reporters distinguished testicular, ovarian, and bipotential gonad-like cell states. Second, mouse ESCs were induced into bipotential cells resembling embryonic gonadal precursors. XY cells were then directed toward a SRY-box transcription factor 9 (Sox9)-positive testicular somatic cell-like cell (TesLC) state by inhibiting bone morphogenetic protein (BMP) and Wntless/Int-1 (Wnt) signaling. Third, TesLCs and PGCLCs were co-aggregated to form testicular organoids. These organoids contained supporting cells, interstitial cells, basement membrane components, and seminiferous tubule-like structures. Fourth, Vasa-tdTomato-positive germ cells were isolated from the organoids and used to establish germline stem cell-like cells (GSCLCs). After transplantation into infertile mouse testes, these cells generated fertilization-competent sperm and fertile offspring^[1]. Thus, the main advance of this model is not the induction of a single cell type. Rather, it reconstructs a developmental sequence in an organoid system. In this sequence, somatic cells

determine sex, the microenvironment guides germ cells, and the *in vivo* niche completes maturation.

2 Molecular mechanism: *in vitro* reconstruction of the sex-determination network

Sex determination depends on the balance between pro-male and pro-female regulatory networks (Figure 1). *In vivo*, sex-determining region Y (Sry) activates Sox9. Downstream factors, including anti-Müllerian hormone (Amh), doublesex and mab-3 related transcription factor 1 (Dmrt1), and fibroblast growth factor 9 (Fgf9), stabilize Sertoli-cell fate. In contrast, R-spondin 1 (Rspo1)/Wnt family member 4 (Wnt4)/β-catenin and BMP signaling promote ovarian fate^[1-3]. Yoshino *et al.*^[1] showed that Wnt and BMP inhibition promoted the formation of Sox9-positive TesLCs. This indicates that male fate requires activation of the Sry-Sox9 axis and suppression of competing pro-ovarian signals.

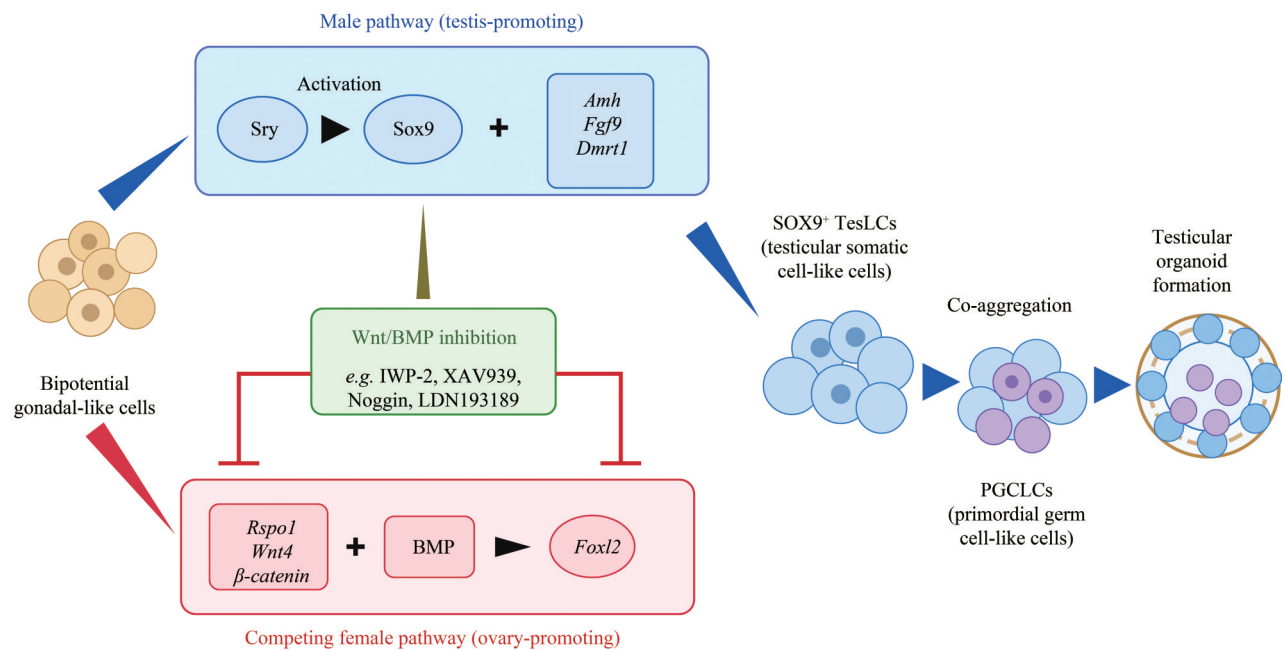


Fig. 1 Schematic illustration of the sex-determination mechanism underlying testicular organoid formation

Testicular organoid formation may then involve three coordinated processes: somatic-cell self-organization, extracellular matrix remodeling, and germline-niche interaction. Sox9-positive Sertoli-like cells may act as organizer cells. They produce anti-Müllerian hormone (AMH), fibroblast growth factor 9

(FGF9), desert hedgehog (DHH), and doublesex and mab-3 related transcription factor 1 (DMRT1)-related signals. Interstitial and peritubular myoid-like cells may support matrix deposition, basement membrane formation, and compartmental separation^[1,4-5]. These cues facilitate PGCLC incorporation and progression

toward prospermatogonia and GSCLCs. However, functional sperm production still requires transplantation. This suggests that vascularization, endocrine gradients, immune barriers, and luminal dynamics are not fully reconstructed *in vitro*^[1].

3 Iteration of reproductive microenvironment models: from tissue culture and directed differentiation to organoids

Previous *in vitro* models of male reproduction include testicular tissue culture, PSC-derived PGCLC differentiation systems, and testicular organoids. Tissue culture preserves native architecture but is limited by tissue availability and experimental controllability^[6]. PSC-derived PGCLC systems are useful for mechanistic studies, yet they lack key niche components, including Sertoli cells, Leydig cells, peritubular myoid cells, extracellular matrix, and basement membrane^[7-9]. Primary-cell-derived testicular organoids can partially recapitulate somatic-germ cell interactions, seminiferous tubule-like structures, endocrine activity, and blood-testis-barrier-related features, but they usually depend on differentiated testicular tissues and cannot model the developmental origin of sex determination^[4-5].

Recent studies have advanced both primary-cell-derived and stem-cell-derived gonadal/testicular organoid models. Primary human or rodent testicular cells can self-organize into organoids with androgen production, human chorionic gonadotropin (hCG) responsiveness, blood-testis-barrier-related properties, and partial support for germ-cell development^[4-5]. Stem-cell-derived models provide a more scalable platform for studying early gonad development. Knarston *et al.*^[10] induced human iPSCs (hiPSCs) into early testis-like cells and organoids expressing bipotential gonadal and Sertoli-cell markers. Pryzhkova *et al.*^[11] established human PSC (hPSC)-derived gonadal organoids for modeling early human gonad development, and Huang *et al.*^[12] generated hiPSC-derived testicular organoids with testicular-cord-like structures, hormone responsiveness, and blood-testis-barrier-related features. Pachernegg *et al.*^[13] further showed that human stem cell-derived testis-like organoids contain multiple testicular lineages, including bipotential gonadal cells, early Sertoli cells, Leydig cells,

peritubular myoid cells, and interstitial cells, and that nuclear receptor subfamily 5 group A member 1 (NR5A1)/steroidogenic factor 1 (SF1) induction promotes Sertoli- and Leydig-cell marker expression.

Compared with these models, Yoshino *et al.*^[1] integrated bipotential gonadal induction, Sry-Sox9-mediated sex determination, testicular somatic cell-like cell (TesLC) generation, PGCLC co-aggregation, GSCLC derivation, and transplantation-based functional validation in one framework. Its main advance lies in linking sex determination, testicular niche assembly, and sperm production from the stage of gonadal specification, rather than simply reassembling pre-existing testicular cells. Nevertheless, the system still relies on transplantation, and its long-term genetic and epigenetic safety requires further evaluation. Therefore, this model is currently more suitable for mechanistic research, disease modeling, and drug screening than for direct reproductive application.

4 Translational prospects, technical bottlenecks, and ethical boundaries

Testicular organoids have broad potential applications in reproductive biology, male infertility research, drug screening, and reproductive toxicology. By reconstructing interactions among germ cells, Sertoli-like cells, Leydig-like cells, peritubular myoid-like cells, the extracellular matrix, and the basement membrane, these models provide controllable systems for studying spermatogenesis and related disorders. Key processes include spermatogonial stem cell (SSC) maintenance, meiotic entry, blood-testis barrier formation, and epigenetic reprogramming^[12,14-15]. Patient-specific iPSC-derived testicular organoids may help model azoospermia, disorders of sex development, and spermatogenic arrest, while distinguishing germ-cell-intrinsic defects from niche-derived abnormalities^[14-17]. They also provide platforms for evaluating candidate drugs, endocrine disruptors, chemotherapeutic agents, metabolic drugs, and environmental toxicants, with endpoints such as Sertoli-cell maturation, Leydig-cell endocrine function, SSC maintenance and haploid-cell formation^[4,12,14]. Integration with hydrogels, microfluidic perfusion, organ-on-chip platforms, and single-cell or spatial multi-omics may further improve physiological relevance and screening efficiency.

Important limitations remain. The current model is mouse-based, whereas human primordial germ cell (PGC) specification, SRY-box transcription factor 17 (SOX17) dependence, gonadal developmental timing, and meiotic progression differ from those in mice^[16]. hPSC-based male germline models mainly reach the human PGCLC (hPGCLC) or pro-spermatogonia-like stage and have not achieved clinically acceptable functional spermatogenesis^[17]. Mature sperm production still depends on the *in vivo* testicular environment after transplantation, indicating that vascularization, hormonal gradients, immune barriers, and luminal dynamics remain incompletely reconstructed *in vitro*^[1]. Germline reprogramming also requires validation beyond marker expression. Although DNA methyltransferase 3 like (DNMT3L), promyelocytic leukemia zinc finger (PLZF), and synaptonemal complex protein 3 (SYCP3) are informative, epigenetic resetting, paternal imprint establishment, transposon silencing, chromosome synapsis, XY-body formation, crossover recombination, and haploid genome stability need further assessment^[18-19]. Therefore, this technology is currently more suitable for disease modeling, mechanistic research, and drug evaluation than for human assisted reproduction. Any future human application will require long-term genetic safety assessment, prohibition of reproductive germline editing, informed consent, sample provenance review, and robust regulatory oversight.

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重建睾丸微环境：干细胞衍生睾丸类器官的机制和转化应用前景*

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摘要 哺乳动物雄性生殖系发育依赖于特化的性腺体细胞微环境。然而, 长期以来, 体外重构这一过程一直受到多重因素限制, 包括难以获得合适的支持细胞谱系、缺乏有序的空间组织结构, 以及培养条件不足以维持生殖细胞成熟等。Yoshino 等建立了一种小鼠多能干细胞来源的睾丸类器官模型, 该模型能够重构由性别决定区域 Y (sex-determining region Y, Sry)-SRY 盒转录因子 9 (SRY-box transcription factor 9, Sox9) 介导的性别决定程序, 并生成睾丸体细胞样细胞 (testicular somatic cell-like cells, TesLCs)。当 TesLCs 与原始生殖细胞样细胞 (primordial germ cell-like cells, PGCLCs) 共同组装时, 可形成类似曲细精管的结构, 并支持雄性生殖系向前精原细胞和生殖干细胞样细胞 (germline stem cell-like cells, GSCLCs) 方向发育。将类器官来源的 GSCLCs 移植至不育受体小鼠睾丸后, 这些细胞能够产生具有受精能力的精子, 并最终获得可育后代。因此, 该研究的核心意义并不仅在于“产生精子”, 而在于将性别决定、生殖细胞命运转换以及睾丸微环境的自组织过程整合到一个可实验操作的类器官系统中。本文将从技术策略、性别决定机制、睾丸微环境重构价值以及转化应用局限性等方面讨论该研究, 并阐明其对体外配子发生、男性不育模型构建和生殖医学基础研究的启示。

关键词 多能干细胞, 性别决定, 睾丸类器官, 体外配子发生, 生殖微环境

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