

## Stem Cell and Testis Study Literatures

Ma Hongbao

Brookdale University Hospital and Medical Center, Brooklyn, New York 11212, USA  
[ma8080@gmail.com](mailto:ma8080@gmail.com)

**Abstract:** The testicle is the male gonad in animals. Like the ovaries to which they are homologous, testes are components of both the reproductive system and the endocrine system. The primary functions of the testes are to produce sperm and to produce androgens, primarily testosterone. Both functions of the testicle are influenced by gonadotropic hormones produced by the anterior pituitary. Luteinizing hormone results in testosterone release. The presence of both testosterone and follicle-stimulating hormone is needed to support spermatogenesis. It has also been shown in animal studies that if testes are exposed to either too high or too low levels of estrogens spermatogenesis can be disrupted to such an extent that the animals become infertile. Stem cells are undifferentiated cells that can divide to more stem cells and differentiate into specialized cells, which exist in multicellular organisms. In mammals, there are two types of stem cells, one is embryonic stem cells (from the inner cell mass of blastocysts) and the other one is adult stem cells (in various tissues). In adult organisms, stem cells and progenitor cells act as a repair system for the body. In a developing embryo, stem cells can differentiate into all the specialized cells. The endocrine pancreas produce insulin. One of the major pancreatic diseases, diabetes mellitus is a metabolic disorder caused by having an insufficient number of insulin-producing  $\beta$  cells. The shortage in donor pancreata could be treated by using alternative sources of stem cells.

[Ma H. **Stem Cell and Testis Study Literatures**. *Stem Cell* 2015;6(1):81-99]. (ISSN 1545-4570).  
<http://www.sciencepub.net/stem>. 11

**Key words:** testis; DNA; life; stem cell; differentiation

### 1. Introduction

The testicle is the male gonad in animals. Like the ovaries to which they are homologous, testes are components of both the reproductive system and the endocrine system. The primary functions of the testes are to produce sperm and to produce androgens, primarily testosterone. Both functions of the testicle are influenced by gonadotropic hormones produced by the anterior pituitary. Luteinizing hormone results in testosterone release. The presence of both testosterone and follicle-stimulating hormone is needed to support spermatogenesis. It has also been shown in animal studies that if testes are exposed to either too high or too low levels of estrogens spermatogenesis can be disrupted to such an extent that the animals become infertile.

Stem cells are undifferentiated cells that can divide to more stem cells and differentiate into specialized cells, which exist in multicellular organisms. In mammals, there are two types of stem cells, one is embryonic stem cells (from the inner cell mass of blastocysts) and the other one is adult stem cells (in various tissues). In adult organisms, stem cells and progenitor cells act as a repair system for the body. In a developing embryo, stem cells can differentiate into all the specialized cells. The endocrine pancreas produce insulin. One of the major pancreatic diseases, diabetes mellitus is a metabolic disorder caused by having an insufficient number of insulin-producing  $\beta$  cells. The shortage in donor pancreata could be treated

by using alternative sources of stem cells. The adult pancreas retains regenerative capacity and it remains unclear whether this organ contains stem cells. Cellular reprogramming or transdifferentiation of exocrine cells or other types of endocrine cells in the pancreas could provide a long-term solution.

The following introduces recent reports as references in the related studies.

Abud, H. E. and G. R. Hime "Regulation of cell adhesion in the testis: a new role for p73." *Asian J Androl* **16**(6): 799-800.

The dramatic changes that male germ cells in the adult testis undergo in gene expression profile and morphology as they transition from spermatogonial stem cells through to mature spermatozoa is dependent upon their association with Sertoli cells. Sertoli cells are crucial for survival and maturation of male germ cells. Two recent papers, Holembowski et al. and Inoue et al. have described a surprising role for the p53 family member, p73, in regulation of germ cell-Sertoli cell adhesion.

Aghamir, S. M., A. Salavati, et al. "Does bone marrow-derived mesenchymal stem cell transfusion prevent antisperm antibody production after traumatic testis rupture?" *Urology* **84**(1): 82-6.

To determine whether transfusion of mesenchymal stem cells (MSCs) could prevent humoral immune response and autoimmunization

against sperms after traumatic testis rupture. Immunomodulatory properties of MSCs have been evaluated by a prospective cohort on 50 adult BALB/c mice. In each interventional arms of study, controlled testis rupture and surgical repair were exerted. In this in vivo model of autoimmune infertility, bone marrow-derived MSC transfusion showed immunosuppressive effects on antibody production. Considering immunomodulatory properties of MSCs even in allogeneic settings, novel clinical application should be investigated further.

Aloisio, G. M., Y. Nakada, et al. "PAX7 expression defines germline stem cells in the adult testis." *J Clin Invest* **124**(9): 3929-44.

Spermatogenesis is a complex, multistep process that maintains male fertility and is sustained by rare germline stem cells. Spermatogenic progression begins with spermatogonia, populations of which express distinct markers. The identity of the spermatogonial stem cell population in the undisturbed testis is controversial due to a lack of reliable and specific markers. Here we identified the transcription factor PAX7 as a specific marker of a rare subpopulation of A(single) spermatogonia in mice.

Amoyel, M., B. D. Simons, et al. "Neutral competition of stem cells is skewed by proliferative changes downstream of Hh and Hpo." *Embo J* **33**(20): 2295-313.

Neutral competition, an emerging feature of stem cell homeostasis, posits that individual stem cells can be lost and replaced by their neighbors stochastically, resulting in chance dominance of a clone at the niche. A single stem cell with an oncogenic mutation could bias this process and clonally spread the mutation throughout the stem cell pool. The *Drosophila* testis provides an ideal system for testing this model. The niche supports two stem cell populations that compete for niche occupancy. Here, we show that cyst stem cells (CySCs) conform to the paradigm of neutral competition and that clonal deregulation of either the Hedgehog (Hh) or Hippo (Hpo) pathway allows a single CySC to colonize the niche.

An, J., X. Zhang, et al. "The histone methyltransferase ESET is required for the survival of spermatogonial stem/progenitor cells in mice." *Cell Death Dis* **5**: e1196.

Self-renewal and differentiation of spermatogonial stem cells (SSCs) are the foundation of spermatogenesis throughout a male's life. SSC transplantation will be a valuable solution for young male patients to preserve their fertility. As SSCs in the collected testis tissue from the patients are very

limited, it is necessary to expansion the SSCs in vitro. Previous studies suggested that histone methyltransferase ERG-associated protein with SET domain (ESET) represses gene expression and is essential for the maintenance of the pool of embryonic stem cells and neurons. The objective of this study was to determine the role of ESET in SSCs using in vitro cell culture and germ cell transplantation. Cell transplantation assay showed that knockdown of ESET reduced the number of seminiferous tubules with spermatogenesis when compared with that of the control.

Azizollahi, S., R. Aflatoonian, et al. "Recruiting testicular torsion introduces an azoospermic mouse model for spermatogonial stem cell transplantation." *Urol J* **11**(3): 1648-55.

To investigate the long-term effect of testicular torsion on sperm parameters and testis structure in order to introduce a novel mice azoospermic model for spermatogonial stem cell transplantation. Unilateral testicular torsion was created. Torsion can cause permanent azoospermia in mouse. Also Testicular torsion 2 weeks after the 2 hours ischemia reperfusion may prove useful for recipient preparation for SSCs transplantation in mouse.

Bellaiche, J., J. J. Lareyre, et al. "Spermatogonial stem cell quest: nanos2, marker of a subpopulation of undifferentiated A spermatogonia in trout testis." *Biol Reprod* **90**(4): 79.

Continuous or cyclic production of spermatozoa throughout life in adult male vertebrates depends on a subpopulation of undifferentiated germ cells acting as spermatogonial stem cells (SSCs). What makes these cells self-renew or differentiate is barely understood, in particular in nonmammalian species, including fish. In the highly seasonal rainbow trout, at the end of the annual spermatogenetic cycle, tubules of the spawning testis contain only spermatozoa, with the exception of scarce undifferentiated spermatogonia that remain on the tubular wall and that will support the next round of spermatogenesis. Taking advantage of this model, we identified putative SSCs in fish testis using morphological, molecular, and functional approaches. In all stages, large spermatogonia with ultrastructural characteristics of germinal stem cells were found, isolated or in doublet. Trout homologues of SSC and/or immature progenitor markers in mammals-nanos2 and nanos3, pou2, plzf, and piwil2-were preferentially expressed in the prepubertal testis and in the undifferentiated A spermatogonia populations purified by centrifugal elutriation. This expression profile strongly suggests that these genes are functionally conserved between fish and mammals.

Moreover, transplantation into embryonic recipients of the undifferentiated spermatogonial cells demonstrated their high "stemness" efficiency in terms of migration into gonads and the ability to give functional gametes.

Busada, J. T., V. A. Chappell, et al. "Retinoic acid regulates Kit translation during spermatogonial differentiation in the mouse." *Dev Biol* **397**(1): 140-9.

In the testis, a subset of spermatogonia retains stem cell potential, while others differentiate to eventually become spermatozoa. This delicate balance must be maintained, as defects can result in testicular cancer or infertility. Currently, little is known about the gene products and signaling pathways directing these critical cell fate decisions. Retinoic acid (RA) is a requisite driver of spermatogonial differentiation and entry into meiosis, yet the mechanisms activated downstream are undefined. Here, we determined a requirement for RA in the expression of KIT, a receptor tyrosine kinase essential for spermatogonial differentiation. We found that RA signaling utilized the PI3K/AKT/mTOR signaling pathway to induce the efficient translation of mRNAs for Kit, which are present but not translated in undifferentiated spermatogonia. Our findings provide an important molecular link between a morphogen (RA) and the expression of KIT protein, which together direct the differentiation of spermatogonia throughout the male reproductive lifespan.

Castle, J. C., M. Loewer, et al. "Immunomic, genomic and transcriptomic characterization of CT26 colorectal carcinoma." *BMC Genomics* **15**: 190.

Tumor models are critical for our understanding of cancer and the development of cancer therapeutics. Here, we present an integrated map of the genome, transcriptome and immunome of an epithelial mouse tumor, the CT26 colon carcinoma cell line. We found that Kras is homozygously mutated at p.G12D, Apc and Tp53 are not mutated, and Cdkn2a is homozygously deleted. Proliferation and stem-cell markers, including Top2a, Birc5 (Survivin), Cldn6 and Mki67, are highly expressed while differentiation and top-crypt markers Muc2, Ms4a8a (MS4A8B) and Epcam are not. Myc, Trp53 (tp53), Mdm2, Hif1a, and Nras are highly expressed while Egfr and Flt1 are not. MHC class I but not MHC class II is expressed. Several known cancer-testis antigens are expressed, including Atad2, Cep55, and Pbk. The highest expressed gene is a mutated form of the mouse tumor antigen gp70. Of the 1,688 non-synonymous point variations, 154 are both in expressed genes and in peptides predicted to bind MHC and thus potential targets for immunotherapy development. Based on its molecular signature, we predicted that CT26 is refractory to anti-EGFR mAbs and sensitive to MEK

and MET inhibitors, as have been previously reported. CT26 cells share molecular features with aggressive, undifferentiated, refractory human colorectal carcinoma cells. As CT26 is one of the most extensively used syngeneic mouse tumor models, our data provide a map for the rationale design of mode-of-action studies for pre-clinical evaluation of targeted- and immunotherapies.

Chan, F., M. J. Oatley, et al. "Functional and molecular features of the Id4+ germline stem cell population in mouse testes." *Genes Dev* **28**(12): 1351-62.

The maintenance of cycling cell lineages relies on undifferentiated subpopulations consisting of stem and progenitor pools. Features that delineate these cell types are undefined for many lineages, including spermatogenesis, which is supported by an undifferentiated spermatogonial population. Here, we generated a transgenic mouse line in which spermatogonial stem cells are marked by expression of an inhibitor of differentiation 4 (Id4)-green fluorescent protein (Gfp) transgene. We found that Id4-Gfp(+) cells exist primarily as a subset of the type A(single) pool, and their frequency is greatest in neonatal development and then decreases in proportion during establishment of the spermatogenic lineage, eventually comprising approximately 2% of the undifferentiated spermatogonial population in adulthood. RNA sequencing analysis revealed that expression of 11 and 25 genes is unique for the Id4-Gfp(+)/stem cell and Id4-Gfp(-)/progenitor fractions, respectively. Collectively, these findings provide the first definitive evidence that stem cells exist as a rare subset of the A(single) pool and reveal transcriptome features distinguishing stem cell and progenitor states within the mammalian male germline.

Chen, S. R. and Y. X. Liu "Regulation of spermatogonial stem cell self-renewal and spermatocyte meiosis by Sertoli cell signaling." *Reproduction* **149**(4): R159-R167.

Spermatogenesis is a continuous and productive process supported by the self-renewal and differentiation of spermatogonial stem cells (SSCs), which arise from undifferentiated precursors known as gonocytes and are strictly controlled in a special 'niche' microenvironment in the seminiferous tubules. Sertoli cells, the only somatic cell type in the tubules, directly interact with SSCs to control their proliferation and differentiation through the secretion of specific factors. Spermatocyte meiosis is another key step of spermatogenesis, which is regulated by Sertoli cells on the luminal side of the blood-testis barrier through paracrine signaling. In this review, we mainly focus on the role of Sertoli cells in the regulation of SSC self-

renewal and spermatocyte meiosis, with particular emphasis on paracrine and endocrine-mediated signaling pathways. Sertoli cell growth factors, such as glial cell line-derived neurotrophic factor (GDNF) and fibroblast growth factor 2 (FGF2), as well as Sertoli cell transcription factors, such as ETS variant 5 (ERM; also known as ETV5), nociceptin, neuregulin 1 (NRG1), and androgen receptor (AR), have been identified as the most important upstream factors that regulate SSC self-renewal and spermatocyte meiosis. Other transcription factors and signaling pathways (GDNF-RET-GFRA1 signaling, FGF2-MAP2K1 signaling, CXCL12-CXCR4 signaling, CCL9-CCR1 signaling, FSH-nociceptin/OPRL1, retinoic acid/FSH-NRG/ERBB4, and AR/RB-ARID4A/ARID4B) are also addressed.

DeFalco, T., I. Bhattacharya, et al. "Yolk-sac-derived macrophages regulate fetal testis vascularization and morphogenesis." *Proc Natl Acad Sci U S A* **111**(23): E2384-93.

Organogenesis of the testis is initiated when expression of Sry in pre-Sertoli cells directs the gonad toward a male-specific fate. The cells in the early bipotential gonad undergo de novo organization to form testis cords that enclose germ cells inside tubules lined by epithelial Sertoli cells. Although Sertoli cells are a driving force in the de novo formation of testis cords, recent studies in mouse showed that reorganization of the vasculature and of interstitial cells also play critical roles in testis cord morphogenesis. However, the mechanism driving reorganization of the vasculature during fetal organogenesis remained unclear. Here we demonstrate that fetal macrophages are associated with nascent gonadal and mesonephric vasculature during the initial phases of testis morphogenesis. Macrophages mediate vascular reorganization and prune errant germ cells and somatic cells after testis architecture is established. We show that gonadal macrophages are derived from primitive yolk-sac hematopoietic progenitors and exhibit hallmarks of M2 activation status, suggestive of angiogenic and tissue remodeling functions. Depletion of macrophages resulted in impaired vascular reorganization and abnormal cord formation. These findings reveal a previously unappreciated role for macrophages in testis morphogenesis and suggest that macrophages are an intermediary between neovascularization and organ architecture during fetal organogenesis.

Dores, C. and I. Dobrinski "De novo morphogenesis of testis tissue: an improved bioassay to investigate the role of VEGF165 during testis formation." *Reproduction* **148**(1): 109-17.

De novo formation of testis tissue from single-cell suspensions allows manipulation of different testicular compartments before grafting to study testicular development and the spermatogonial stem cell niche. However, the low percentages of newly formed seminiferous tubules supporting complete spermatogenesis and lack of a defined protocol have limited the use of this bioassay. Low spermatogenic efficiency in de novo formed tissue could result from the scarcity of germ cells in the donor cell suspension, cell damage caused by handling or from hypoxia during tissue formation in the host environment.

Esteves, C. L., R. Sharma, et al. "Expression of putative markers of pluripotency in equine embryonic and adult tissues." *Vet J* **202**(3): 533-5.

Expression of several putative markers of pluripotency (OCT4, SOX2, NANOG, LIN28A, REX1, DNMT3B and TERT) was examined in a range of equine tissues, including early embryos, induced pluripotent stem cells (iPSCs), testis, adipose- and bone marrow-derived mesenchymal stromal cells (MSCs), and keratinocytes. Transcript levels of all markers were highest in embryos and iPSCs and, except for SOX2, were very low or undetectable in keratinocytes. Mean expression levels of all markers were lower in testis than in embryos or iPSCs and, except for DNMT3B, were higher in testis than in MSCs. Expression of OCT4, NANOG and DNMT3B, but not the other markers, was detected in MSCs. Of all markers analysed, only LIN28A, REX1 and TERT were associated exclusively with pluripotent cells in the horse.

Eun, S. H., Z. Shi, et al. "A non-cell autonomous role of E(z) to prevent germ cells from turning on a somatic cell marker." *Science* **343**(6178): 1513-6.

In many metazoans, germ cells are separated from somatic lineages early in development and maintain their identity throughout life. Here, we show that a Polycomb group (PcG) component, Enhancer of Zeste [E(z)], a histone transferase that generates trimethylation at lysine 27 of histone H3, maintains germline identity in *Drosophila* adult testes. We find excessive early-stage somatic gonadal cells in E(z) mutant testes, which originate from both overproliferative cyst stem cells and germ cells turning on an early-stage somatic cell marker. Using complementary lineage-tracing experiments in E(z) mutant testes, a portion of excessive early-stage somatic gonadal cells are found to originate from early-stage germ cells, including germline stem cells. Moreover, knocking down E(z) specifically in somatic cells caused this change, which suggests a non-cell



autonomous role of E(z) to antagonize somatic identity in germ cells.

Figueira, M. I., H. J. Cardoso, et al. "Hormonal regulation of c-KIT receptor and its ligand: implications for human infertility?" Prog Histochem Cytochem **49**(1-3): 1-19.

The c-KIT, a tyrosine kinase receptor, and its ligand the stem cell factor (SCF) play an important role in the production of male and female gametes. The interaction of SCF with c-KIT is required for germ cell survival and growth, and abnormalities in the activity of the SCF/c-KIT system have been associated with human infertility. Recently, it was demonstrated that gonadotropic and sex steroid hormones, among others, regulate the expression of SCF and c-KIT in testicular and ovarian cells. Therefore, the hormonal (de)regulation of SCF/c-KIT system in the testis and ovary may be a cause underpinning infertility. In the present review, we will discuss the effects of hormones modulating the expression levels of SCF and c-KIT in the human gonads. In addition, the implications of hormonal regulation of SCF/c-KIT system for germ cell development and fertility will be highlighted.

Flenkenthaler, F., S. Windschuttl, et al. "Secretome analysis of testicular peritubular cells: a window into the human testicular microenvironment and the spermatogonial stem cell niche in man." J Proteome Res **13**(3): 1259-69.

Spermatogonial stem cells (SSCs) are vital for lifelong spermatogenesis in man. In their niches, a special growth factor milieu and structural support by surrounding cells are thought to ensure their maintenance. In man, the cells of the wall of seminiferous tubules, human testicular peritubular cells (HTPCs), are considered to contribute to this microenvironment and the overall testicular microenvironment via secreted proteins. Therefore, the secretome of cultured HTPCs from five individual men was analyzed by LC-MS/MS. Quantification and comparison to the proteome of HTPC lysates revealed 263 out of 660 identified secretome proteins to be at least 5-fold enriched in the culture media. To obtain additional evidence for secretion, signal peptide and gene ontology (GO) enrichment analyses were applied. The latter revealed--besides extracellular matrix (ECM) components--a significant over-representation of chemokines and growth factors acting in signaling pathways that appear critical for SSC maintenance. Immunohistochemistry, performed with human testicular sections, depicted expression of selected proteins in vivo. The significant enrichment of proteins related to cell adhesion and migration may indicate their involvement in SSC regulation. Our data

strongly support the hypothesis of a crucial role of HTPCs in the composition of SSC niches in man.

Garcia, T. X., J. K. Farmaha, et al. "RBPJ in mouse Sertoli cells is required for proper regulation of the testis stem cell niche." Development **141**(23): 4468-78.

Stem cells are influenced by their surrounding microenvironment, or niche. In the testis, Sertoli cells are the key niche cells directing the population size and differentiation fate of spermatogonial stem cells (SSCs). Failure to properly regulate SSCs leads to infertility or germ cell hyperplasia. Several Sertoli cell-expressed genes, such as Gdnf and Cyp26b1, have been identified as being indispensable for the proper maintenance of SSCs in their niche, but the pathways that modulate their expression have not been identified. Although we have recently found that constitutively activating NOTCH signaling in Sertoli cells leads to premature differentiation of all prospermatogonia and sterility, suggesting that there is a crucial role for this pathway in the testis stem cell niche, a true physiological function of NOTCH signaling in Sertoli cells has not been demonstrated. To this end, we conditionally ablated recombination signal binding protein for immunoglobulin kappa J region (Rbpj), a crucial mediator of NOTCH signaling, in Sertoli cells using Amh-cre. Rbpj knockout mice had: significantly increased testis sizes; increased expression of niche factors, such as Gdnf and Cyp26b1; significant increases in the number of pre- and post-meiotic germ cells, including SSCs; and, in a significant proportion of mice, testicular failure and atrophy with tubule lithiasis, possibly due to these unsustainable increases in the number of germ cells. We also identified germ cells as the NOTCH ligand-expressing cells. We conclude that NOTCH signaling in Sertoli cells is required for proper regulation of the testis stem cell niche and is a potential feedback mechanism, based on germ cell input, that governs the expression of factors that control SSC proliferation and differentiation.

Gartner, S. M., C. Rathke, et al. "Ex vivo culture of Drosophila pupal testis and single male germ-line cysts: dissection, imaging, and pharmacological treatment." J Vis Exp(91): 51868.

During spermatogenesis in mammals and in *Drosophila melanogaster*, male germ cells develop in a series of essential developmental processes. This includes differentiation from a stem cell population, mitotic amplification, and meiosis. In addition, post-meiotic germ cells undergo a dramatic morphological reshaping process as well as a global epigenetic reconfiguration of the germ line chromatin--the histone-to-protamine switch. Studying the role of a

protein in post-meiotic spermatogenesis using mutagenesis or other genetic tools is often impeded by essential embryonic, pre-meiotic, or meiotic functions of the protein under investigation. The post-meiotic phenotype of a mutant of such a protein could be obscured through an earlier developmental block, or the interpretation of the phenotype could be complicated. The model organism *Drosophila melanogaster* offers a bypass to this problem: intact testes and even cysts of germ cells dissected from early pupae are able to develop *ex vivo* in culture medium. Making use of such cultures allows microscopic imaging of living germ cells in testes and of germ-line cysts. Importantly, the cultivated testes and germ cells also become accessible to pharmacological inhibitors, thereby permitting manipulation of enzymatic functions during spermatogenesis, including post-meiotic stages. The protocol presented describes how to dissect and cultivate pupal testes and germ-line cysts. Information on the development of pupal testes and culture conditions are provided alongside microscope imaging data of live testes and germ-line cysts in culture. We also describe a pharmacological assay to study post-meiotic spermatogenesis, exemplified by an assay targeting the histone-to-protamine switch using the histone acetyltransferase inhibitor anacardic acid. In principle, this cultivation method could be adapted to address many other research questions in pre- and post-meiotic spermatogenesis.

Ginsberg, J. P., Y. Li, et al. "Testicular tissue cryopreservation in prepubertal male children: an analysis of parental decision-making." *Pediatr Blood Cancer* **61**(9): 1673-8.

**BACKGROUND:** Infertility is an unfortunate treatment-related consequence for some pediatric malignancies as well as some non-malignant conditions treated with stem cell transplant. Unlike pubertal males, prepubertal males cannot produce semen for cryopreservation. This manuscript reports on the acceptability and safety of a multi-institutional protocol for offering testicular tissue cryopreservation to families of prepubertal male children at highest risk for infertility. Data on decision influences, decision-making control, and emotional state when considering this option are described. **PROCEDURE:** Prepubertal males facing gonadotoxic therapy were offered testicular cryopreservation. Post-biopsy, patients were followed for acute side effects. In addition, parents and patients were asked to complete questionnaires, whether or not they chose to cryopreserve tissue. **RESULTS:** Seventy-four prepubertal male children were approached. Fifty-seven families (77%) consented to the testicular biopsy; 48 of 57 underwent the procedure. There was one post-operative side

effect. Parents who agreed to testicular cryopreservation and those that did not felt in control of this decision. Parents who consented to the biopsy and refusers were not deterred by the experimental nature of the protocol. An important decision-making influence was the risk of the biopsy. **CONCLUSION:** Biopsy and cryopreservation of testicular tissue from prepubertal male children was performed successfully and safely at three institutions. Parents faced with this option at diagnosis can make an informed decision and weigh carefully the risks and benefits. Although asked to make a decision soon after they were given a difficult diagnosis, parents uniformly felt in control of this decision.

Goossens, E. and H. Tournaye "Male fertility preservation, where are we in 2014?" *Ann Endocrinol (Paris)* **75**(2): 115-7.

Male fertility preservation receives growing attention in the field of reproductive medicine. The first clinical programs were established to preserve reproductive potential in men needing gonadotoxic treatment. Sperm cryopreservation is now a standard procedure. Since a few years, several centres offer testicular tissue cryopreservation to prepubertal boys. This method is still experimental and further research is needed to implement the transplantation techniques in the clinic. With the aim to preserve or restore fertility in patients affected by other diseases (Klinefelter syndrome, Sertoli cell only syndrome), techniques for *in vitro* spermatogenesis are being developed.

Habert, R., V. Muczynski, et al. "Concerns about the widespread use of rodent models for human risk assessments of endocrine disruptors." *Reproduction* **147**(4): R119-29.

Fetal testis is a major target of endocrine disruptors (EDs). During the last 20 years, we have developed an organotypic culture system that maintains the function of the different fetal testis cell types and have used this approach as a toxicological test to evaluate the effects of various compounds on gametogenesis and steroidogenesis in rat, mouse and human testes. We named this test rat, mouse and human fetal testis assay. With this approach, we compared the effects of six potential EDs ((mono-(2-ethylhexyl) phthalate (MEHP), cadmium, depleted uranium, diethylstilboestrol (DES), bisphenol A (BPA) and metformin) and one signalling molecule (retinoic acid (RA)) on the function of rat, mouse and human fetal testis at a comparable developmental stage. We found that the response is similar in humans and rodents for only one third of our analyses. For instance, RA and MEHP have similar negative effects on gametogenesis in the three species. For another

third of our analyses, the threshold efficient concentrations that disturb gametogenesis and/or steroidogenesis differ as a function of the species. For instance, BPA and metformin have similar negative effects on steroidogenesis in human and rodents, but at different threshold doses. For the last third of our analyses, the qualitative response is species specific. For instance, MEHP and DES affect steroidogenesis in rodents, but not in human fetal testis. These species differences raise concerns about the extrapolation of data obtained in rodents to human health risk assessment and highlight the need of rigorous comparisons of the effects in human and rodent models, when assessing ED risk.

Hara, K., T. Nakagawa, et al. "Mouse spermatogenic stem cells continually interconvert between equipotent singly isolated and syncytial states." *Cell Stem Cell* **14**(5): 658-72.

The identity and behavior of mouse spermatogenic stem cells have been a long-standing focus of interest. In the prevailing "As model," stem cell function is restricted to singly isolated (As) spermatogonia. By examining single-cell dynamics of GFRalpha1+ stem cells *in vivo*, we evaluate an alternative hypothesis that, through fragmentation, syncytial spermatogonia also contribute to stem cell function in homeostasis. We use live imaging and pulse labeling to quantitatively determine the fates of individual GFRalpha1+ cells and find that, during steady-state spermatogenesis, the entire GFRalpha1+ population comprises a single stem cell pool, in which cells continually interconvert between As and syncytial states. A minimal biophysical model, relying only on the rates of incomplete cell division and syncytial fragmentation, precisely predicts the stochastic fates of GFRalpha1+ cells during steady state and postinsult regeneration. Thus, our results define an alternative and dynamic model for spermatogenic stem cell function in the mouse testis.

Hartung, O., M. M. Forbes, et al. "Zebrafish vasa is required for germ-cell differentiation and maintenance." *Mol Reprod Dev* **81**(10): 946-61.

Vasa is a universal marker of the germ line in animals, yet mutations disrupting vasa cause sexually dimorphic infertility, with impaired development of the ovary in some animals and the testis in others. The basis for this sexually dimorphic requirement for Vasa is not clear; in most animals examined, both the male and female gonad express vasa throughout the life of the germ line. Here we characterized a loss-of-function mutation disrupting zebrafish vasa. We show that maternally provided Vasa is stable through the first ten days of development in zebrafish, and thus likely fulfills any early roles for Vasa during germ-line

specification, migration, survival, and maintenance. Although zygotic Vasa is not essential for the development of juvenile gonads, vasa mutants develop exclusively as sterile males. Furthermore, phenotypes of vasa;p53 compound mutants are indistinguishable from those of vasa mutants, therefore the failure of vasa mutants to differentiate as females and to support germ-cell development in the testis is not due to p53-mediated apoptosis. Instead, we found that failure to progress beyond the pachytene stage of meiosis causes the loss of germ-line stem cells, leaving empty somatic tubules. Our studies provide insight into the function of zebrafish vasa during female meiosis, differentiation, and maintenance of germ-line stem cells.

Hasegawa, K. and Y. Saga "FGF8-FGFR1 signaling acts as a niche factor for maintaining undifferentiated spermatogonia in the mouse." *Biol Reprod* **91**(6): 145.

In mammalian testes, spermatogonial stem cells (SSCs) maintain spermatogenesis over a long period of time by undergoing self-renewal and differentiation. SSCs are among the most primitive of spermatogenic cells (undifferentiated spermatogonia), and their activities are strictly regulated by extrinsic niche factors. However, the factors that constitute a testicular niche remain poorly understood. In this study, we demonstrate that fibroblast growth factor (FGF) signaling maintains undifferentiated spermatogonia through activating ERK1/2 signaling *in vivo*. Undifferentiated spermatogonia comprise GFRA1(+) and NANOS3(+) subpopulations, which are likely to undergo self-renewal and enter the differentiation pathway, respectively. In the testis, Fgfr1 was expressed in the entire population of undifferentiated spermatogonia, and deleting FGFR1 in spermatogenic cells partially inactivated ERK1/2 and resulted in reduced numbers of both GFRA1(+) and NANOS3(+) cells. In addition, Fgf8 was expressed in spermatogenic cells, and loss- and gain-of-function models of FGF8 demonstrated that FGF8 positively regulated the numbers of undifferentiated spermatogonia through FGFR1, particularly among NANOS3(+) cells. Finally we show a possible involvement of FGF signaling in the reversion from NANOS3(+) into GFRA1(+) undifferentiated spermatogonia. Taken together, our data suggest that FGF signaling is an important component of the testicular niche and has a unique function for maintaining undifferentiated spermatogonia.

Hayashi, M., M. Sato, et al. "Enrichment of spermatogonial stem cells using side population in teleost." *Biol Reprod* **91**(1): 23.

Spermatogenesis originates from a small population of spermatogonial stem cells; this

population can maintain continuous sperm production throughout the life of fish via self-renewal and differentiation. Despite their biological importance, spermatogonial stem cells are not thoroughly characterized because they are difficult to distinguish from their progeny cells that become committed to differentiation. We previously established a novel technique for germ cell transplantation to identify spermatogonial stem cells based on their colonizing activity and their ability to initiate donor-derived gametogenesis in the rainbow trout (*Oncorhynchus mykiss*). Although spermatogonial stem cells can be retrospectively identified after transplantation, there is currently no technique to prospectively enrich for or purify spermatogonial stem cells. Here, we describe a method for spermatogonial stem cell enrichment using a side population. With optimized Hoechst 33342 staining conditions, we successfully identified side-population cells among type A spermatogonia. Side-population cells were transcriptomically and morphologically distinct from non-side-population cells. To functionally determine whether the transplantable spermatogonial stem cells were enriched in the side-population fraction, we compared the colonization activity of side-population cells with that of non-side-population cells. Colonization efficiency was significantly higher with side-population cells than with non-side-population cells or with total type A spermatogonia. In addition, side-population cells could produce billions of sperm in recipients. These results indicated that transplantable spermatogonial stem cells were enriched in the side-population fraction. This method will provide biological information that may advance our understanding of spermatogonial stem cells in teleosts. Additionally, this technique will increase the efficiency of germ cell transplantation used in surrogate broodstock technology.

Heidari, B., M. Gifani, et al. "Enrichment of undifferentiated type A spermatogonia from goat testis using discontinuous percoll density gradient and differential plating." *Avicenna J Med Biotechnol* 6(2): 94-103.

**BACKGROUND:** The well documented source for adult multipotent stem cells is Spermatogonial Stem Cells (SSCs). They are the foundation of spermatogenesis in the testis throughout adult life by balancing self-renewal and differentiation. The aim of this study was to assess the effect of percoll density gradient and differential plating on enrichment of undifferentiated type A spermatogonia in dissociated cellular suspension of goat testes. Additionally, we evaluated the separated fractions of the gradients in percoll and samples in differential plating at different times for cell number, viability and

purification rate of goat SSCs in culture. **METHODS:** Testicular cells were successfully isolated from one month old goat testis using two-step enzymatic digestion and followed by two purification protocols, differential plating with different times of culture (3, 4, 5, and 6 hr) and discontinuous percoll density with different gradients (20, 28, 30, and 32%). The difference of percentage of undifferentiated SSCs (PGP9.5 positive) in each method was compared using ANOVA and comparison between the highest percentage of corresponding value between two methods was carried out by t-test using Sigma Stat (ver. 3.5). **RESULTS:** The highest PGP9.5 (94.6±0.4) and the lowest c-Kit positive (25.1±0.7) in Percoll method was significantly ( $p \leq 0.001$ ) achieved in 32% percoll gradient. While the corresponding rates in differential plating method for the highest PGP9.5 positive cells (81.3±1.1) and lowest c-Kit (17.1±1.4) was achieved after 5 hr culturing ( $p < 0.001$ ). The enrichment of undifferentiated type A spermatogonia using Percoll was more efficient than differential plating method ( $p < 0.001$ ). **CONCLUSION:** Percoll density gradient and differential plating were efficient and fast methods for enrichment of type A spermatogonial stem cells from goat testes.

Hetie, P., M. de Cuevas, et al. "Conversion of quiescent niche cells to somatic stem cells causes ectopic niche formation in the *Drosophila* testis." *Cell Rep* 7(3): 715-21.

Adult stem cells reside in specialized regulatory microenvironments, or niches, where local signals ensure stem cell maintenance. The *Drosophila* testis contains a well-characterized niche wherein signals from postmitotic hub cells promote maintenance of adjacent germline stem cells and somatic cyst stem cells (CySCs). Hub cells were considered to be terminally differentiated; here, we show that they can give rise to CySCs. Genetic ablation of CySCs triggers hub cells to transiently exit quiescence, delaminate from the hub, and convert into functional CySCs. Ectopic Cyclin D-Cdk4 expression in hub cells is also sufficient to trigger their conversion into CySCs. In both cases, this conversion causes the formation of multiple ectopic niches over time. Therefore, our work provides a model for understanding how oncogenic mutations in quiescent niche cells could promote loss of quiescence, changes in cell fate, and aberrant niche expansion.

Ishii, K., M. Kanatsu-Shinohara, et al. "Cell-cycle-dependent colonization of mouse spermatogonial stem cells after transplantation into seminiferous tubules." *J Reprod Dev* 60(1): 37-46.



Spermatogonial stem cells (SSCs) migrate to the niche upon introduction into the seminiferous tubules of the testis of infertile animals. However, only 5-10% of the transplanted cells colonize recipient testes. In this study, we analyzed the impact of cell cycle on spermatogonial transplantation. We used fluorescent ubiquitination-based cell cycle indicator transgenic mice to examine the influence of cell cycle on SSC activity of mouse germline stem (GS) cells, a population of cultured spermatogonia enriched for SSCs. GS cells in the G1 phase are more efficient than those in the S/G2-M phase in colonizing the seminiferous tubules of adult mice. Cells in the G1 phase not only showed higher expression levels of GFRA1, a component of the GDNF self-renewal factor receptor, but also adhered more efficiently to laminin-coated plates. Furthermore, this cell cycle-dependency was not observed when cells were transplanted into immature pup recipients, which do not have the blood-testis barrier (BTB) between Sertoli cells, suggesting that cells in the G1 phase may pass through the BTB more readily than cells in the S/G2-M phase. Thus cell cycle status is an important factor in regulating SSC migration to the niche.

Jankovic Velickovic, L. and V. Stefanovic "Hypoxia and spermatogenesis." *Int Urol Nephrol* **46**(5): 887-94.

This review mainly focuses on our understanding of spermatogenesis in physiological and pathological hypoxic condition. Real hypoxia is closely related to vascular changes and an increase in testicular temperature. Both induce a reduction in sperm count and can be related to the increase in germ cell apoptosis. On the other hand, change in the temperature, and oxygen levels in the microenvironment have influence on spermatogonial stem cell function and differentiation. The initial connection between hypoxia and a factor critical for stem cell maintenance is alteration in Oct-4 expression, and these data may be a useful strategy for modulating stem cell function. Unilateral testicular ischemia-induced cell death can be accompanied by an increase in germ cell apoptosis in the contralateral testis. The injury of contralateral testis following unilateral testicular damage is controversial, and it can contribute to the reduction in fertility.

Jiang, M. H., B. Cai, et al. "Characterization of Nestin-positive stem Leydig cells as a potential source for the treatment of testicular Leydig cell dysfunction." *Cell Res* **24**(12): 1466-85.

The ability to identify and isolate lineage-specific stem cells from adult tissues could facilitate cell replacement therapy. Leydig cells (LCs) are the primary source of androgen in the mammalian testis, and the prospective identification of stem Leydig cells

(SLCs) may offer new opportunities for treating testosterone deficiency. Here, in a transgenic mouse model expressing GFP driven by the Nestin (Nes) promoter, we observed Nes-GFP+ cells located in the testicular interstitial compartment where SLCs normally reside. We showed that these Nes-GFP+ cells expressed LIFR and PDGFR-alpha, but not LC lineage markers. We further observed that these cells were capable of clonogenic self-renewal and extensive proliferation in vitro and could differentiate into neural or mesenchymal cell lineages, as well as LCs, with the ability to produce testosterone, under defined conditions. Moreover, when transplanted into the testes of LC-disrupted or aging models, the Nes-GFP+ cells colonized the interstitium and partially increased testosterone production, and then accelerated meiotic and post-meiotic germ cell recovery. In addition, we further demonstrated that CD51 might be a putative cell surface marker for SLCs, similar with Nestin. Taken together, these results suggest that Nes-GFP+ cells from the testis have the characteristics of SLCs, and our study would shed new light on developing stem cell replacement therapy for testosterone deficiency.

Kakiuchi, K., A. Tsuda, et al. "Cell-surface DEAD-box polypeptide 4-immunoreactive cells and gonocytes are two distinct populations in postnatal porcine testes." *Biol Reprod* **90**(4): 82.

DEAD-box polypeptide 4 (DDX4) is an evolutionally conserved ATP-dependent RNA helicase that is exclusively expressed in germ cell lineage. Although DDX4 is believed to reside and function in the cytoplasm, recent studies in mice and humans suggest that its epitope is expressed on the cell surface of a small subpopulation in the ovary, putative oogonial stem cells. No study has examined whether such cell-surface DDX4(+) cells exist in the testes of any species. In this study, we explored cell-surface DDX4(+) cells in postnatal porcine testes before the onset of spermatogenesis, where gonocytes, which are the precursors of spermatogonial stem cells, are the only germ cell population. Transfection experiments demonstrated that recombinant porcine DDX4 can be expressed on the cell surface, and cell-surface DDX4-immunoreactive cells were identified in the testis by flow cytometry. Although the DDX4-expressing cells identified in the testis were indeed gonocytes, the cell-surface DDX4-immunoreactive cells expressed negligible DDX4 mRNA and protein levels. Furthermore, they did not express other germ cell markers, such as ZBTB16, NANOS2, and DAZL, but prominently expressed early primordial germ cell markers, such as PRDM1, IFITM3, and EPCAM. Nonetheless, the cell-surface DDX4-immunoreactive cells generated neither germ cell colonies nor

teratomas following transplantation into immunocompromised mouse testes. Taken together, these results demonstrate that testicular cell-surface DDX4-immunoreactive cells are not germ cells and constitute a distinct subpopulation that is different from gonocytes. Moreover, the subpopulation in porcine testes might be species specific because no DDX4-immunoreactive cells were found in postnatal mouse testes.

Kanatsu-Shinohara, M., N. Ogonuki, et al. "Improved serum- and feeder-free culture of mouse germline stem cells." *Biol Reprod* **91**(4): 88.

Spermatogonial stem cells (SSCs) undergo self-renewal division, which can be recapitulated in vitro. Attempts to establish serum-free culture conditions for SSCs have met with limited success. Although we previously reported that SSCs can be cultured without serum on laminin-coated plates, the growth rate and SSC concentration were relatively low, which made it inefficient for culturing large numbers of SSCs. In this study, we report on a novel culture medium that showed improved SSC maintenance. We used Iscove modified Dulbecco medium, supplemented with lipid mixture, fetuin, and knockout serum replacement. In the presence of glial cell line-derived neurotrophic factor (GDNF) and fibroblast growth factor 2 (FGF2), SSCs cultured on laminin-coated plates could proliferate for more than 5 mo and maintained normal karyotype and androgenetic DNA methylation patterns in imprinted genes. Germ cell transplantation showed that SSCs in the serum-free medium proliferated more actively than those in the serum-supplemented medium and that the frequency of SSCs was comparable between the two culture media. Cultured cells underwent germline transmission. Development of a new serum- and feeder-free culture method for SSCs will facilitate studies into the effects of microenvironments on self-renewal and will stimulate further improvements to derive SSC cultures from different animal species.

Kanatsu-Shinohara, M., I. Onoyama, et al. "Skp1-Cullin-F-box (SCF)-type ubiquitin ligase FBXW7 negatively regulates spermatogonial stem cell self-renewal." *Proc Natl Acad Sci U S A* **111**(24): 8826-31.

Spermatogonial stem cells (SSCs) undergo self-renewal divisions to support spermatogenesis throughout life. Although several positive regulators of SSC self-renewal have been discovered, little is known about the negative regulators. Here, we report that F-box and WD-40 domain protein 7 (FBXW7), a component of the Skp1-Cullin-F-box-type ubiquitin ligase, is a negative regulator of SSC self-renewal. FBXW7 is expressed in undifferentiated spermatogonia in a cell cycle-dependent manner.

Although peptidyl-prolyl cis/trans isomerase NIMA-interacting 1 (PIN1), essential for spermatogenesis, is thought to destroy FBXW7, Pin1 depletion decreased FBXW7 expression. Spermatogonial transplantation showed that Fbxw7 overexpression compromised SSC activity whereas Fbxw7 deficiency enhanced SSC colonization and caused accumulation of undifferentiated spermatogonia, suggesting that the level of FBXW7 is critical for self-renewal and differentiation. Screening of putative FBXW7 targets revealed that Fbxw7 deficiency up-regulated myelocytomatosis oncogene (MYC) and cyclin E1 (CCNE1). Although depletion of Myc/Mycn or Ccne1/Ccne2 compromised SSC activity, overexpression of Myc, but not Ccne1, increased colonization of SSCs. These results suggest that FBXW7 regulates SSC self-renewal in a negative manner by degradation of MYC.

Kumar, T. R. "The quest for male germline stem cell markers: PAX7 gets ID'd." *J Clin Invest* **124**(10): 4219-22.

Male germline or spermatogonial stem cells (SSCs) are conserved across many species and essential for uninterrupted production of sperm over long periods of reproductive life span. A better understanding of SSC biology provides limitless opportunities in male reproductive health, fertility preservation, and regenerative medicine. Although several potential markers define SSCs, not many definitive markers exist that are specific for a rare subset of SSCs that self-renew and have the ability to give rise to other progenitors, eventually contributing to all stages of spermatogenesis. In the September 2014 issue of the JCI, Aloisio and colleagues report that PAX7 is a new marker expressed uniquely in a rare subset of SSCs in mouse testes. PAX7+ cells fulfill all the criteria required for bona fide SSCs. Surprisingly, male germline-specific deletion of Pax7 indicates that it is dispensable for spermatogenesis.

Lee, W. Y., K. H. Lee, et al. "Transcriptional coactivator undifferentiated embryonic cell transcription factor 1 expressed in spermatogonial stem cells: a putative marker of boar spermatogonia." *Anim Reprod Sci* **150**(3-4): 115-24.

Spermatogenesis is initiated from spermatogonial stem cells (SSCs), which are derived from gonocytes. Although some rodent SSC markers have been investigated, other species- and developmental stage-specific markers of spermatogonia have not been identified. The objective of this study was to characterize the expression of undifferentiated embryonic cell transcription factor 1 (UTF1) gene as a potential marker for spermatogonia and SSCs in the boar testis. In boar testis tissue at pre-

pubertal stages (tissues collected at 5, 30, and 60 days of age), UTF1 gene expression was detected in almost all spermatogonia cells that expressed a protein gene product 9.5 (PGP9.5), and immunocytochemical analysis of isolated total testicular cells showed that 91.14% of cells staining for PGP9.5 also stained for UTF1. However, in boar testis tissue at pubertal and post-pubertal stages (tissues collected at 90, 120, 150, and 180 days of age), UTF1 was not detected in all PGP9.5-positive cells in the basement membrane. While some PGP9.5-positive cells stained for UTF1, other cells stained only for PGP9.5 or UTF1. PGP9.5, UTF1, and NANOG was assessed in *in vitro* cultures of pig SSCs (pSSCs) from testes collected at 5 days of age. The relative amounts of PGP9.5, NANOG, and UTF1 mRNA were greater in pSSC colonies than in testis and muscle tissue. Thus, the UTF1 gene is expressed in PGP9.5-positive spermatogonia cells of pigs at 5 days of age, and its expression is maintained in cultured pSSC colonies, suggesting that UTF1 is a putative marker for early-stage spermatogonia in the pre-pubertal pig testis. These findings will facilitate the study of spermatogenesis and applications in germ cell research.

Lee, Y. A., Y. H. Kim, et al. "Effect of sugar molecules on the cryopreservation of mouse spermatogonial stem cells." *Fertil Steril* **101**(4): 1165-75 e5.

**OBJECTIVE:** To study the influence of sugars and establish a serum-free freezing method for the cryopreservation of spermatogonial stem cells (SSCs). **DESIGN:** Animal study. **SETTING:** University laboratory. **ANIMAL(S):** C57BL/6-TgEGFP, C57BL/6 mice. **INTERVENTION(S):** Germ cells enriched from testis cells were frozen using standard freezing medium containing sugars, including monosaccharides, disaccharides, and trisaccharides at 50, 100, and 200 mM, respectively. To study the feasibility of establishing a serum-free freezing method, fetal bovine serum was substituted with knockout serum replacement. **MAIN OUTCOME MEASURE(S):** Freeze-thawed germ cells were evaluated for recovery rate, proliferation capacity, and stem cell activity after transplantation to recipient testes. **RESULT(S):** Supplementation of freezing medium with 200 mM disaccharide is an effective method for cryopreservation of SSCs. Trehalose is the most effective cryoprotectant among all the sugars tested and only lactose was comparable to trehalose. Our proliferation and transplantation data show that serum-free freezing can be achieved in freezing medium supplemented with 200 mM trehalose, 10% knockout serum replacement, and 10% dimethyl sulfoxide (DMSO) for cryopreservation of SSCs. **CONCLUSION(S):** These findings raise the

possibility of effectively banking frozen SSCs from various species, including humans, in a traditional serum-free medium for germ cell research and male infertility treatments.

Lee, Y. A., Y. H. Kim, et al. "Cryopreservation of porcine spermatogonial stem cells by slow-freezing testis tissue in trehalose." *J Anim Sci* **92**(3): 984-95.

Spermatogonial stem cells provide the foundation for continued adult spermatogenesis and their manipulation can facilitate assisted reproductive technologies or the development of transgenic animals. Because the pig is an important agricultural and biomedical research animal, the development of practical application techniques to manipulate the pig Spermatogonial stem cell is needed. The ability to preserve porcine Spermatogonial stem cell or testis tissue long term is one of these fundamental techniques. The objective of this study was to optimize methods to cryopreserve porcine Spermatogonial stem cell when freezing testis cells or testis tissue. To identify the most efficient cryopreservation technique, porcine testis cells (cell freezing) or testis tissue (tissue freezing) were frozen in medium containing dimethyl sulfoxide (DMSO) and fetal bovine serum (FBS) or DMSO, FBS, and various concentrations of trehalose (50, 100, or 200 mM). After thawing, undifferentiated germ cells were enriched and treatments were evaluated for cryopreservation efficiency. The tissue freezing method resulted in significantly greater germ cell recovery ( $P = 0.041$ ) and proliferation capacity ( $P < 0.001$ ) compared to the cell freezing treatment. Regardless of freezing method (cell vs. tissue), addition of 200 mM trehalose to freezing medium increased germ cell recovery and proliferation capacity compared to cells frozen using the same freezing method without trehalose. Interestingly, addition of trehalose to the tissue freezing medium significantly increased germ cell recovery ( $P = 0.012$ ) and proliferation capacity ( $P = 0.004$ ) compared to the cell freezing treatment supplemented with trehalose. To confirm that cryopreservation in trehalose improves the survival of Spermatogonial stem cell, testis cells enriched for undifferentiated germ cells were xenotransplanted into recipient mouse testes. Germ cells recovered from tissue frozen with 200 mM trehalose generated significantly more ( $P < 0.001$ ) donor derived colonies than tissue frozen without trehalose. Regardless of cryopreservation medium or freezing method, testis cell recovery, viability, and proliferation capacity of germ cells after thawing were significantly lower compared to those of untreated fresh control. Nevertheless, these data demonstrate that undifferentiated porcine germ cells can be efficiently cryopreserved in the presence of 200 mM trehalose.

Lim, J. J., D. W. Seol, et al. "Spermatogonial stem cell enrichment using simple grafting of testis and in vitro cultivation." *Sci Rep* **4**: 5923.

Enrichment of spermatogonial stem cells (SSCs) from the mammalian adult testis faces several limitations owing to their relatively low numbers among many types of advanced germ cells and somatic cells. The aim of the present study was to improve the isolation efficiency of SSCs using a simple tissue grafting method to eliminate the existing advanced germ cells. Sliced testis parenchyma obtained from adult ICR or EGFP-expressing transgenic mice were grafted heterotopically under the dorsal skin of nude mice. The most advanced germ cells disappeared in the grafted tissues after 2-4 weeks. Grafted tissues were dissociated enzymatically and plated in culture dishes. During in vitro culture, significantly more SSCs were obtained from the grafted testes than from non-grafted controls, and the isolated SSCs had proliferative potential and were successfully maintained. Additionally, EGFP-expressing SSCs derived from graft parenchyma were transplanted into bulsufan-treated recipient mice testes. Finally, we obtained EGFP-expressing pups after in vitro fertilization using spermatozoa derived from transplanted SSCs. These results suggest that subcutaneous grafting of testis parenchyma and the subsequent culture methods provide a simple and efficient isolation method to enrich for SSCs in adult testis without specific cell sorting methods and may be useful tools for clinical applications.

Naillat, F., W. Yan, et al. "Identification of the genes regulated by Wnt-4, a critical signal for commitment of the ovary." *Exp Cell Res* **332**(2): 163-78.

The indifferent mammalian embryonic gonad generates an ovary or testis, but the factors involved are still poorly known. The Wnt-4 signal represents one critical female determinant, since its absence leads to partial female-to-male sex reversal in mouse, but its signalling is as well implicated in the testis development. We used the Wnt-4 deficient mouse as a model to identify candidate gonadogenesis genes, and found that the *Notum*, *Phlda2*, *Runx-1* and *Msx1* genes are typical of the wild-type ovary and the *Osr2*, *Dach2*, *Pitx2* and *Tacr3* genes of the testis. Strikingly, the expression of these latter genes becomes reversed in the Wnt-4 knock-out ovary, suggesting a role in ovarian development. We identified the transcription factor *Runx-1* as a Wnt-4 signalling target gene, since it is expressed in the ovary and is reduced upon Wnt-4 knock-out. Consistent with this, introduction of the Wnt-4 signal into early ovary cells ex vivo induces *Runx-1* expression, while conversely Wnt-4 expression is down-regulated in the absence of *Runx-*

1. We conclude that the *Runx-1* gene can be a Wnt-4 signalling target, and that *Runx-1* and Wnt-4 are mutually interdependent in their expression. The changes in gene expression due to the absence of Wnt-4 in gonads reflect the sexually dimorphic role of this signal and its complex gene network in mammalian gonad development.

Nakajima, S., M. Hayashi, et al. "Expression patterns of *gdnf* and *gfralpha1* in rainbow trout testis." *Gene Expr Patterns* **14**(2): 111-20.

In mice, glial cell line-derived neurotrophic factor (GDNF) is essential for normal spermatogenesis and in vitro culture of spermatogonial stem cells. In murine testes, GDNF acts as paracrine factor; Sertoli cells secrete it to a subset of spermatogonial cells expressing its receptor, GDNF family receptor alpha1 (GFRalpha1). However, in fish, it is unclear what types of cells express *gdnf* and *gfralpha1*. In this study, we isolated the rainbow trout orthologues of these genes and analyzed their expression patterns during spermatogenesis. In rainbow trout testes, *gdnf* and *gfralpha1* were expressed in almost all type A spermatogonia (ASG). Noticeably, unlike in mice, the expression of *gdnf* was not observed in Sertoli cells in rainbow trout. During spermatogenesis, the expression levels of these genes changed synchronously; *gdnf* and *gfralpha1* showed high expression in ASG and decreased dramatically in subsequent developmental stages. These results suggested that GDNF most likely acts as an autocrine factor in rainbow trout testes.

Nazm Bojnordi, M., M. Movahedin, et al. "Oligoprogenitor cells derived from spermatogonia stem cells improve remyelination in demyelination model." *Mol Biotechnol* **56**(5): 387-93.

Embryonic stem (ES) like cells-derived testis represents a possible alternative to replace of neurons and glia. Here, we differentiated spermatogonia cells to oligoprogenitor (OP) like cells and transplanted them to demyelination model and assess their recovery potential in a demyelinated corpus callosum model in rats. ES like cells were differentiated to OP like cells using appropriate inducers and were transplanted in situ to demyelinated corpus callosum. Cell integration as well as demyelination extension and myelination intensity changes were evaluated using histologic studies and immunocytochemistry after 2 and 4 weeks post transplantation. Investigation of Nestin, NF68, Olig2, and NG2 by immunocytochemical technique indicated the differentiation of ES like cells to neuroprogenitor and oligodendrocyte like cells in each induction stage. Histologic findings showed a significant decrease in demyelination extension and a significant increase in remyelination intensity in cell transplanted groups. Also on the base of PLP



expression, differentiation of transplanted cells was confirmed to myelinogenic cells using immunocytochemistry technique. We conclude that ES like cells derived from spermatogonia cells can be differentiated to OP like cells that can form myelin after transplantation into the demyelination model in rat, this represents recovery potential of spermatogonia cells which opens new window for cell therapeutic approaches using spermatogonial stem cells.

Odeh, H. M., C. Kleinguetl, et al. "Regulation of the proliferation and differentiation of Leydig stem cells in the adult testis." *Biol Reprod* **90**(6): 123.

We reported previously that stem cells associated with adult rat testis seminiferous tubules are able to give rise to differentiated Leydig cells in vitro. The regulatory mechanisms by which they do so, however, are uncertain. Herein, we hypothesized that the proliferation and differentiation of Leydig cell stem cells (stem Leydig cells, SLCs) depend upon locally produced factors from the seminiferous tubules. Microarray analysis revealed that platelet-derived growth factor receptor alpha (PDGFRalpha) is up-regulated and PDGFRbeta is down-regulated with postnatal differentiation of SLCs. This suggested that their ligands, PDGF-AA and PDGF-BB, respectively, might have important roles in SLC proliferation and differentiation. To test this, we developed a unique in vitro culture system in which SLCs proliferate on the surfaces of cultured seminiferous tubules largely during Week 1 of culture and their progeny subsequently differentiate to testosterone-forming Leydig cells during Weeks 2 through 4. Using this system, seminiferous tubules from adult rat testes were cultured with PDGF-AA or PDGF-BB for up to 4 wk. Both ligands stimulated SLC proliferation during the first week of culture, with PDGF-BB significantly more potent than PDGF-AA. Furthermore, PDGF-AA had a stimulatory effect on SLC differentiation from Weeks 2 through 4 of culture. In contrast, PDGF-BB, which stimulated cell proliferation during Week 1, had a significant inhibitory effect on differentiation during Weeks 2 through 4. These findings, made possible by the development of the seminiferous tubule culture system, reveal distinct roles by locally produced PDGFs in SLC regulation.

Papagiannouli, F. and I. Lohmann "Stage-specific control of stem cell niche architecture in the *Drosophila* testis by the posterior Hox gene Abd-B." *Comput Struct Biotechnol J* **13**: 122-30.

A fundamental question in biology is how complex structures are maintained after their initial specification. We address this question by reviewing the role of the Hox gene Abd-B in *Drosophila* testis organogenesis, which proceeds through embryonic,

larval and pupal stages to reach maturation in adult stages. The data presented in this review highlight a cell- and stage-specific function of Abd-B, since the mechanisms regulating stem cell niche positioning and architecture at different stages seem to be different despite the employment of similar factors. In addition to its described role in the male embryonic gonads, sustained activity of Abd-B in the pre-meiotic germline spermatocytes during larval stages is required to maintain the architecture of the stem cell niche by regulating betaPS-integrin localization in the neighboring somatic cyst cells. Loss of Abd-B is associated with cell non-autonomous effects within the niche, leading to a dramatic reduction of pre-meiotic cell populations in adult testes. Identification of Abd-B target genes revealed that Abd-B mediates its effects by controlling the activity of the sevenless ligand Boss via its direct targets Src42A and Sec63. During adult stages, when testis morphogenesis is completed with the addition of the acto-myosin sheath originating from the genital disc, stem cell niche positioning and integrity are regulated by Abd-B activity in the acto-myosin sheath whereas integrin acts in an Abd-B independent way. It seems that the occurrence of new cell types and cell interactions in the course of testis organogenesis made it necessary to adapt the system to the new cellular conditions by reusing the same players for testis stem cell niche positioning in an alternative manner.

Qian, Y., C. L. Ng, et al. "CSN maintains the germline cellular microenvironment and controls the level of stem cell genes via distinct CRLs in testes of *Drosophila melanogaster*." *Dev Biol* **398**(1): 68-79.

Stem cells and their daughters are often associated with and depend on cues from their cellular microenvironment. In *Drosophila* testes, each Germline Stem Cell (GSC) contacts apical hub cells and is enclosed by cytoplasmic extensions from two Cyst Stem Cells (CySCs). Each GSC daughter becomes enclosed by cytoplasmic extensions from two CySC daughters, called cyst cells. CySC fate depends on an Unpaired (Upd) signal from the hub cells, which activates the Janus Kinase and Signal Transducer and Activator of Transcription (Jak/STAT) pathway in the stem cells. Germline enclosure depends on Epidermal Growth Factor (EGF) signals from the germline to the somatic support cells. Expression of RNA-hairpins against subunits of the CONstitutively Photomorphogenic-9- (COP9-) signalosome (CSN) in somatic support cells disrupted germline enclosure. Furthermore, CSN-depleted somatic support cells in the CySC position next to the hub had reduced levels of the Jak/STAT effectors Zinc finger homeotic-1 (Zfh-1) and Chronologically inappropriate morphogenesis (Chinmo). Knockdown of CSN in the

somatic support cells does not disrupt EGF and Upd signal transduction as downstream signal transducers, phosphorylated STAT (pSTAT) and phosphorylated Mitogen Activated Protein Kinase (pMAPK), were still localized to the somatic support cell nuclei. The CSN modifies fully formed Cullin RING ubiquitin ligase (CRL) complexes to regulate selective proteolysis. Reducing cullin2 (cul2) from the somatic support cells disrupted germline enclosure, while reducing cullin1 (cul1) from the somatic support cells led to a low level of Chinmo. We propose that different CRLs enable the responses of somatic support cells to Upd and EGF.

Ramm, S. A. and L. Scharer "The evolutionary ecology of testicular function: size isn't everything." *Biol Rev Camb Philos Soc* **89**(4): 874-88.

Larger testes are considered the quintessential adaptation to sperm competition. However, the strong focus on testis size in evolutionary research risks ignoring other potentially adaptive features of testicular function, many of which will also be shaped by post-mating sexual selection. Here we advocate a more integrated research programme that simultaneously takes into account the developmental machinery of spermatogenesis and the various selection pressures that act on this machinery and its products. The testis is a complex organ, and so we begin by outlining how we can think about the evolution of testicular function both in terms of the composition and spatial organisation of the testis ('testicular histology'), as well as in terms of the logical organisation of cell division during spermatogenesis ('testicular architecture'). We then apply these concepts to ask which aspects of testicular function we can expect to be shaped by post-mating sexual selection. We first assess the impact of selection on those traits most strongly associated with sperm competition, namely the number and kind of sperm produced. A broad range of studies now support our contention that post-mating sexual selection affects many aspects of testicular function besides gross testis size, for example, to maximise spermatogenic efficiency or to enable the production of particular sperm morphologies. We then broaden our focus to ask how testicular function is affected by fluctuation in sperm demand. Such fluctuation can occur over an individual's lifetime (for example due to seasonality in reproduction) and may select for particular types of testicular histology and architecture depending on the particular reproductive ecology of the species in question. Fluctuation in sperm demand also occurs over evolutionary time, due to shifts in the mating system, and this may have various consequences for testicular function, for example on rates of proliferation-induced mutation and for dealing with

intragenomic conflict. We end by suggesting additional approaches that could be applied to study testicular function, and conclude that simultaneously considering the machinery, products and scheduling of spermatogenesis will be crucial as we seek to understand more fully the evolution of this most fundamental of male reproductive traits.

Sachs, C., B. D. Robinson, et al. "Evaluation of candidate spermatogonial markers ID4 and GPR125 in testes of adult human cadaveric organ donors." *Andrology* **2**(4): 607-14.

The optimal markers for human spermatogonial stem cells (SSCs) are not known. Among the genes recently linked to SSCs in mice and other animals are the basic helix-loop-helix transcription factor ID4 and the orphan G-protein-coupled receptor GPR125. While ID4 and GPR125 are considered putative markers for SSCs, they have not been evaluated for coexpression in human tissue. Furthermore, neither the size nor the character of the human spermatogonial populations that express ID4 and GPR125, respectively, are known. A major barrier to addressing these questions is the availability of healthy adult testis tissue from donors with no known reproductive health problems. To overcome this obstacle, we have employed healthy testicular tissue from a novel set of organ donors (n = 16; aged 17-68 years) who were undergoing post-mortem clinical organ procurement. Using immunolabelling, we found that ID4 and GPR125 are expressed on partially overlapping spermatogonial populations and are more broadly expressed in the normal adult human testis. In addition, we found that expression of ID4 remained stable during ageing. These findings suggest that ID4 and GPR125 could be efficacious for identifying previously unrecognized human spermatogonial subpopulations in conjunction with other putative human stem cell markers, both in younger and older donors.

Sadri-Ardekani, H. and A. Atala "Testicular tissue cryopreservation and spermatogonial stem cell transplantation to restore fertility: from bench to bedside." *Stem Cell Res Ther* **5**(3): 68.

Male infertility management has made significant progress during the past three decades, especially after the introduction of intracytoplasmic sperm injection in 1992. However, many boys and men still suffer from primary testicular failure due to acquired or genetic causes. New and novel treatments are needed to address these issues. Spermatogenesis originates from spermatogonial stem cells (SSCs) that reside in the testis. Many of these men lack SSCs or have lost SSCs over time as a result of specific medical conditions or toxic exposures. Loss of SSCs is

critical in prepubertal boys who suffer from cancer and are going through gonadotoxic cancer treatments, as there is no option of sperm cryopreservation due to sexual immaturity. The development of SSC transplantation in a mouse model to repopulate spermatozoa in depleted testes has opened new avenues of research in other animal models, including non-human primates. Recent advances in cryopreservation and in vitro propagation of human SSCs offer promise for human SSC autotransplantation in the near future. Ongoing research is focusing on safety and technical issues of human SSC autotransplantation. This is the time to counsel parents and boys at risk of infertility on the possibility of cryopreserving and banking a small amount of testis tissue for potential future use in SSC transplantation.

Sakurai, K., I. Talukdar, et al. "Kinome-wide functional analysis highlights the role of cytoskeletal remodeling in somatic cell reprogramming." *Cell Stem Cell* **14**(4): 523-34.

The creation of induced pluripotent stem cells (iPSCs) from somatic cells by ectopic expression of transcription factors has galvanized the fields of regenerative medicine and developmental biology. Here, we report a kinome-wide RNAi-based analysis to identify kinases that regulate somatic cell reprogramming to iPSCs. We prepared 3,686 small hairpin RNA (shRNA) lentiviruses targeting 734 kinase genes covering the entire mouse kinome and individually examined their effects on iPSC generation. We identified 59 kinases as barriers to iPSC generation and characterized seven of them further. We found that shRNA-mediated knockdown of the serine/threonine kinases TESK1 or LIMK2 promoted mesenchymal-to-epithelial transition, decreased COFILIN phosphorylation, and disrupted Actin filament structures during reprogramming of mouse embryonic fibroblasts. Similarly, knockdown of TESK1 in human fibroblasts also promoted reprogramming to iPSCs. Our study reveals the breadth of kinase networks regulating pluripotency and identifies a role for cytoskeletal remodeling in modulating the somatic cell reprogramming process.

Shapiro-Kulnane, L., A. E. Smolko, et al. "Maintenance of *Drosophila* germline stem cell sexual identity in oogenesis and tumorigenesis." *Development* **142**(6): 1073-82.

Adult stem cells maintain tissue homeostasis by balancing self-renewal and differentiation. In *Drosophila* females, germline stem cells (GSCs) require Sex lethal (Sxl) to exit the stem cell state and to enter the differentiation pathway. Without Sxl GSCs do not differentiate and instead form tumors. Previous

studies have shown that these tumors are not caused by a failure in the self-renewal/differentiation switch. Here, we show that Sxl is also necessary for the cell-autonomous maintenance of germ cell female identity and demonstrate that tumors are caused by the acquisition of male characteristics. Germ cells without Sxl protein exhibit a global derepression of testis genes, including Phf7, a male germline sexual identity gene. Phf7 is a key effector of the tumor-forming pathway, as it is both necessary and sufficient for tumor formation. In the absence of Sxl protein, inappropriate Phf7 expression drives tumor formation through a cell-autonomous mechanism that includes sex-inappropriate activation of Jak/Stat signaling. Remarkably, tumor formation requires a novel response to external signals emanating from the GSC niche, highlighting the importance of interactions between mutant cells and the surrounding normal cells that make up the tumor microenvironment. Derepression of testis genes, and inappropriate Phf7 expression, is also observed in germ cell tumors arising from the loss of bag of marbles (bam), demonstrating that maintenance of female sexual identity requires the concerted actions of Sxl and bam. Our work reveals that GSCs must maintain their sexual identity as they are reprogrammed into a differentiated cell, or risk tumorigenesis.

Slaidina, M. and R. Lehmann "Translational control in germline stem cell development." *J Cell Biol* **207**(1): 13-21.

Stem cells give rise to tissues and organs during development and maintain their integrity during adulthood. They have the potential to self-renew or differentiate at each division. To ensure proper organ growth and homeostasis, self-renewal versus differentiation decisions need to be tightly controlled. Systematic genetic studies in *Drosophila melanogaster* are revealing extensive regulatory networks that control the switch between stem cell self-renewal and differentiation in the germline. These networks, which are based primarily on mutual translational repression, act via interlocked feedback loops to provide robustness to this important fate decision.

Stine, R. R., L. J. Greenspan, et al. "Coordinate regulation of stem cell competition by Slit-Robo and JAK-STAT signaling in the *Drosophila* testis." *PLoS Genet* **10**(11): e1004713.

Stem cells in tissues reside in and receive signals from local microenvironments called niches. Understanding how multiple signals within niches integrate to control stem cell function is challenging. The *Drosophila* testis stem cell niche consists of somatic hub cells that maintain both germline stem cells and somatic cyst stem cells (CySCs). Here, we

show a role for the axon guidance pathway Slit-Roundabout (Robo) in the testis niche. The ligand Slit is expressed specifically in hub cells while its receptor, Roundabout 2 (Robo2), is required in CySCs in order for them to compete for occupancy in the niche. CySCs also require the Slit-Robo effector Abelson tyrosine kinase (Abl) to prevent over-adhesion of CySCs to the niche, and CySCs mutant for Abl outcompete wild type CySCs for niche occupancy. Both Robo2 and Abl phenotypes can be rescued through modulation of adherens junction components, suggesting that the two work together to balance CySC adhesion levels. Interestingly, expression of Robo2 requires JAK-STAT signaling, an important maintenance pathway for both germline and cyst stem cells in the testis. Our work indicates that Slit-Robo signaling affects stem cell function downstream of the JAK-STAT pathway by controlling the ability of stem cells to compete for occupancy in their niche.

Takashima, S., M. Kanatsu-Shinohara, et al. "Functional Differences between GDNF-Dependent and FGF2-Dependent Mouse Spermatogonial Stem Cell Self-Renewal." *Stem Cell Reports* 4(3): 489-502.

Spermatogonial stem cells (SSCs) are required for spermatogenesis. Earlier studies showed that glial cell line-derived neurotrophic factor (GDNF) was indispensable for SSC self-renewal by binding to the GFRA1/RET receptor. Mice with mutations in these molecules showed impaired spermatogenesis, which was attributed to SSC depletion. Here we show that SSCs undergo GDNF-independent self-renewal. A small number of spermatogonia formed colonies when testis fragments from a Ret mutant mouse strain were transplanted into heterologous recipients. Moreover, fibroblast growth factor 2 (FGF2) supplementation enabled in vitro SSC expansion without GDNF. Although GDNF-mediated self-renewal signaling required both AKT and MAP2K1/2, the latter was dispensable in FGF2-mediated self-renewal. FGF2-depleted testes exhibited increased levels of GDNF and were enriched for SSCs, suggesting that the balance between FGF2 and GDNF levels influences SSC self-renewal in vivo. Our results show that SSCs exhibit at least two modes of self-renewal and suggest complexity of SSC regulation in vivo.

Tzung, K. W., R. Goto, et al. "Early depletion of primordial germ cells in zebrafish promotes testis formation." *Stem Cell Reports* 4(1): 61-73.

As complete absence of germ cells leads to sterile males in zebrafish, we explored the relationship between primordial germ cell (PGC) number and sexual development. Our results revealed dimorphic proliferation of PGCs in the early zebrafish larvae, marking the beginning of sexual differentiation. We

applied morpholino-based gene knockdown and cell transplantation strategies to demonstrate that a threshold number of PGCs is required for the stability of ovarian fate. Using histology and transcriptomic analyses, we determined that zebrafish gonads are in a meiotic ovarian stage at 14 days postfertilization and identified signaling pathways supporting meiotic oocyte differentiation and eventual female fate. The development of PGC-depleted gonads appears to be restrained and delayed, suggesting that PGC number may directly regulate the variability and length of gonadal transformation and testicular differentiation in zebrafish. We propose that gonadal transformation may function as a developmental buffering mechanism to ensure the reproductive outcome.

van den Driesche, S., R. M. Sharpe, et al. "Regulation of the germ stem cell niche as the foundation for adult spermatogenesis: a role for miRNAs?" *Semin Cell Dev Biol* 29: 76-83.

Within the testis the spermatogonial stem cells reside in a unique microenvironment, or 'niche', which includes the surrounding somatic cells. The regulation of the balance between self-renewal and differentiation of spermatogonial stem cells determines the lifelong supply of spermatozoa by maintaining a population of undifferentiated spermatogonial stem cells and ensuring that adequate numbers of spermatogonia undergo spermatogenesis. Mouse models have been instrumental in determining a large number of factors involved in regulating the spermatogonial stem cell self-renewal and/or differentiation. However, the precise mechanisms controlling regulation of the germ cell niche remain to be elucidated. Recently the discovery of microRNAs, which regulate gene expression at the post-transcriptional level, has provided new insight into testis biology, spermatogenesis and germ stem cell regulation. In this review we summarize the main factors involved in the regulation of the germ stem cell niche and describe the role of microRNA signaling in this regulation.

Voog, J., S. L. Sandall, et al. "Escargot restricts niche cell to stem cell conversion in the Drosophila testis." *Cell Rep* 7(3): 722-34.

Stem cells reside within specialized microenvironments, or niches, that control many aspects of stem cell behavior. Somatic hub cells in the Drosophila testis regulate the behavior of cyst stem cells (CySCs) and germline stem cells (GSCs) and are a primary component of the testis stem cell niche. The shutoff (shof) mutation, characterized by premature loss of GSCs and CySCs, was mapped to a locus encoding the evolutionarily conserved transcription factor Escargot (Esg). Hub cells depleted of Esg



acquire CySC characteristics and differentiate as cyst cells, resulting in complete loss of hub cells and eventually CySCs and GSCs, similar to the shof mutant phenotype. We identified Esg-interacting proteins and demonstrate an interaction between Esg and the corepressor C-terminal binding protein (CtBP), which was also required for maintenance of hub cell fate. Our results indicate that niche cells can acquire stem cell properties upon removal of a single transcription factor in vivo.

Vrooman, L. A., J. M. Oatley, et al. "Estrogenic exposure alters the spermatogonial stem cells in the developing testis, permanently reducing crossover levels in the adult." *PLoS Genet* **11**(1): e1004949.

Bisphenol A (BPA) and other endocrine disrupting chemicals have been reported to induce negative effects on a wide range of physiological processes, including reproduction. In the female, BPA exposure increases meiotic errors, resulting in the production of chromosomally abnormal eggs. Although numerous studies have reported that estrogenic exposures negatively impact spermatogenesis, a direct link between exposures and meiotic errors in males has not been evaluated. To test the effect of estrogenic chemicals on meiotic chromosome dynamics, we exposed male mice to either BPA or to the strong synthetic estrogen, ethinyl estradiol during neonatal development when the first cells initiate meiosis. Although chromosome pairing and synapsis were unperturbed, exposed outbred CD-1 and inbred C3H/HeJ males had significantly reduced levels of crossovers, or meiotic recombination (as defined by the number of MLH1 foci in pachytene cells) by comparison with placebo. Unexpectedly, the effect was not limited to cells exposed at the time of meiotic entry but was evident in all subsequent waves of meiosis. To determine if the meiotic effects induced by estrogen result from changes to the soma or germline of the testis, we transplanted spermatogonial stem cells from exposed males into the testes of unexposed males. Reduced recombination was evident in meocytes derived from colonies of transplanted cells. Taken together, our results suggest that brief exogenous estrogenic exposure causes subtle changes to the stem cell pool that result in permanent alterations in spermatogenesis (i.e., reduced recombination in descendent meocytes) in the adult male.

Xie, W., J. Sun, et al. "Construction and analysis of a protein-protein interaction network related to self-renewal of mouse spermatogonial stem cells." *Mol Biosyst* **11**(3): 835-43.

Spermatogonial stem cells (SSCs) are responsible for sustained spermatogenesis throughout

the reproductive life of the male. Extensive studies of SSCs have identified dozens of genes that play important roles in sustaining or controlling the pool of SSCs in the mammalian testis. However, there is still limited knowledge of whether or how these key genes interact with each other during SSC self-renewal. Here, we constructed a protein-protein interaction (PPI) network for SSC self-renewal based on interactions between 23 genes essential for SSC self-renewal, which were obtained from a text mining system, and the interacting partners of the 23 key genes, which were differentially expressed in SSCs. The SSC self-renewal PPI network consisted of 246 nodes connected by 844 edges. Topological analyses of the PPI network were conducted to identify genes essential for maintenance of SSC self-renewal. The subnetwork of the SSC self-renewal network suggested that the 23 key genes involved in SSC self-renewal were connected together through other 94 genes. Clustering of the whole network and subnetwork of SSC self-renewal revealed several densely connected regions, implying significant molecular interaction modules essential for SSC self-renewal. Notably, we found the 23 genes to be responsible for SSC self-renewal by forming a continuous PPI network centered on Pou5f1. Our study indicates that it is feasible to explore important proteins and regulatory pathways in biological activities by combining a PPI database with the high-throughput data of gene expression profiles.

The above contents are the collected information from Internet and public resources to offer to the people for the convenient reading and information disseminating and sharing.

## References

1. Abud, H. E. and G. R. Hime "Regulation of cell adhesion in the testis: a new role for p73." *Asian J Androl* **16**(6): 799-800.
2. Aghamir, S. M., A. Salavati, et al. "Does bone marrow-derived mesenchymal stem cell transfusion prevent antisperm antibody production after traumatic testis rupture?" *Urology* **84**(1): 82-6.
3. Aloisio, G. M., Y. Nakada, et al. "PAX7 expression defines germline stem cells in the adult testis." *J Clin Invest* **124**(9): 3929-44.
4. Amoyel, M., B. D. Simons, et al. "Neutral competition of stem cells is skewed by proliferative changes downstream of Hh and Hpo." *Embo J* **33**(20): 2295-313.
5. An, J., X. Zhang, et al. "The histone methyltransferase ESET is required for the survival of spermatogonial stem/progenitor cells in mice." *Cell Death Dis* **5**: e1196.
6. Azizollahi, S., R. Aflatoonian, et al. "Recruiting testicular torsion introduces an azoospermic mouse

- model for spermatogonial stem cell transplantation." Urol J **11**(3): 1648-55.
7. Busada, J. T., V. A. Chappell, et al. "Retinoic acid regulates Kit translation during spermatogonial differentiation in the mouse." Dev Biol **397**(1): 140-9.
  8. Castle, J. C., M. Loewer, et al. "Immunomic, genomic and transcriptomic characterization of CT26 colorectal carcinoma." BMC Genomics **15**: 190.
  9. Chan, F., M. J. Oatley, et al. "Functional and molecular features of the Id4+ germline stem cell population in mouse testes." Genes Dev **28**(12): 1351-62.
  10. Chen, S. R. and Y. X. Liu "Regulation of spermatogonial stem cell self-renewal and spermatocyte meiosis by Sertoli cell signaling." Reproduction **149**(4): R159-R167.
  11. DeFalco, T., I. Bhattacharya, et al. "Yolk-sac-derived macrophages regulate fetal testis vascularization and morphogenesis." Proc Natl Acad Sci U S A **111**(23): E2384-93.
  12. Does, C. and I. Dobrinski "De novo morphogenesis of testis tissue: an improved bioassay to investigate the role of VEGF165 during testis formation." Reproduction **148**(1): 109-17.
  13. Esteves, C. L., R. Sharma, et al. "Expression of putative markers of pluripotency in equine embryonic and adult tissues." Vet J **202**(3): 533-5.
  14. Eun, S. H., Z. Shi, et al. "A non-cell autonomous role of E(z) to prevent germ cells from turning on a somatic cell marker." Science **343**(6178): 1513-6.
  15. Figueira, M. I., H. J. Cardoso, et al. "Hormonal regulation of c-KIT receptor and its ligand: implications for human infertility?" Prog Histochem Cytochem **49**(1-3): 1-19.
  16. Flenkenthaler, F., S. Windschuttl, et al. "Secretome analysis of testicular peritubular cells: a window into the human testicular microenvironment and the spermatogonial stem cell niche in man." J Proteome Res **13**(3): 1259-69.
  17. Garcia, T. X., J. K. Farmaha, et al. "RBPJ in mouse Sertoli cells is required for proper regulation of the testis stem cell niche." Development **141**(23): 4468-78.
  18. Gartner, S. M., C. Rathke, et al. "Ex vivo culture of Drosophila pupal testis and single male germ-line cysts: dissection, imaging, and pharmacological treatment." J Vis Exp(91): 51868.
  19. Ginsberg, J. P., Y. Li, et al. "Testicular tissue cryopreservation in prepubertal male children: an analysis of parental decision-making." Pediatr Blood Cancer **61**(9): 1673-8.
  20. Goossens, E. and H. Tournaye "Male fertility preservation, where are we in 2014?" Ann Endocrinol (Paris) **75**(2): 115-7.
  21. Habert, R., V. Muczynski, et al. "Concerns about the widespread use of rodent models for human risk assessments of endocrine disruptors." Reproduction **147**(4): R119-29.
  22. Hara, K., T. Nakagawa, et al. "Mouse spermatogenic stem cells continually interconvert between equipotent singly isolated and syncytial states." Cell Stem Cell **14**(5): 658-72.
  23. Hartung, O., M. M. Forbes, et al. "Zebrafish vasa is required for germ-cell differentiation and maintenance." Mol Reprod Dev **81**(10): 946-61.
  24. Hasegawa, K. and Y. Saga "FGF8-FGFR1 signaling acts as a niche factor for maintaining undifferentiated spermatogonia in the mouse." Biol Reprod **91**(6): 145.
  25. Hayashi, M., M. Sato, et al. "Enrichment of spermatogonial stem cells using side population in teleost." Biol Reprod **91**(1): 23.
  26. Heidari, B., M. Gifani, et al. "Enrichment of undifferentiated type a spermatogonia from goat testis using discontinuous percoll density gradient and differential plating." Avicenna J Med Biotechnol **6**(2): 94-103.
  27. Hetie, P., M. de Cuevas, et al. "Conversion of quiescent niche cells to somatic stem cells causes ectopic niche formation in the Drosophila testis." Cell Rep **7**(3): 715-21.
  28. Ishii, K., M. Kanatsu-Shinohara, et al. "Cell-cycle-dependent colonization of mouse spermatogonial stem cells after transplantation into seminiferous tubules." J Reprod Dev **60**(1): 37-46.
  29. Jankovic Velickovic, L. and V. Stefanovic "Hypoxia and spermatogenesis." Int Urol Nephrol **46**(5): 887-94.
  30. Jiang, M. H., B. Cai, et al. "Characterization of Nestin-positive stem Leydig cells as a potential source for the treatment of testicular Leydig cell dysfunction." Cell Res **24**(12): 1466-85.
  31. Kakiuchi, K., A. Tsuda, et al. "Cell-surface DEAD-box polypeptide 4-immunoreactive cells and gonocytes are two distinct populations in postnatal porcine testes." Biol Reprod **90**(4): 82.
  32. Kanatsu-Shinohara, M., I. Onoyama, et al. "Skp1-Cullin-F-box (SCF)-type ubiquitin ligase FBXW7 negatively regulates spermatogonial stem cell self-renewal." Proc Natl Acad Sci U S A **111**(24): 8826-31.
  33. Kanatsu-Shinohara, M., N. Ogonuki, et al. "Improved serum- and feeder-free culture of mouse germline stem cells." Biol Reprod **91**(4): 88.
  34. Kumar, T. R. "The quest for male germline stem cell markers: PAX7 gets ID'd." J Clin Invest **124**(10): 4219-22.
  35. Lee, W. Y., K. H. Lee, et al. "Transcriptional coactivator undifferentiated embryonic cell transcription factor 1 expressed in spermatogonial stem cells: a putative marker of boar spermatogonia." Anim Reprod Sci **150**(3-4): 115-24.

36. Lee, Y. A., Y. H. Kim, et al. "Cryopreservation of porcine spermatogonial stem cells by slow-freezing testis tissue in trehalose." *J Anim Sci* **92**(3): 984-95.
37. Lee, Y. A., Y. H. Kim, et al. "Effect of sugar molecules on the cryopreservation of mouse spermatogonial stem cells." *Fertil Steril* **101**(4): 1165-75 e5.
38. Lim, J. J., D. W. Seol, et al. "Spermatogonial stem cell enrichment using simple grafting of testis and in vitro cultivation." *Sci Rep* **4**: 5923.
39. Ma H, Chen G. Stem cell. *The Journal of American Science* 2005;1(2):90-92.
40. Ma H, Cherg S. Eternal Life and Stem Cell. *Nature and Science*. 2007;5(1):81-96.
41. Ma H, Cherg S. Nature of Life. *Life Science Journal* 2005;2(1):7 - 15.
42. Ma H, Yang Y. Turritopsis nutricula. *Nature and Science* 2010;8(2):15-20. [http://www.sciencepub.net/nature/ns0802/03\\_1279\\_hongbao\\_turritopsis\\_ns0802\\_15\\_20.pdf](http://www.sciencepub.net/nature/ns0802/03_1279_hongbao_turritopsis_ns0802_15_20.pdf).
43. Ma H. The Nature of Time and Space. *Nature and science* 2003;1(1):1-11. *Nature and science* 2007;5(1):81-96.
44. Naillat, F., W. Yan, et al. "Identification of the genes regulated by Wnt-4, a critical signal for commitment of the ovary." *Exp Cell Res* **332**(2): 163-78.
45. Nakajima, S., M. Hayashi, et al. "Expression patterns of gdnf and gfral in rainbow trout testis." *Gene Expr Patterns* **14**(2): 111-20.
46. National Center for Biotechnology Information, U.S. National Library of Medicine. <http://www.ncbi.nlm.nih.gov/pubmed>. 2015.
47. Nazm Bojnordi, M., M. Movahedin, et al. "Oligoprogenitor cells derived from spermatogonia stem cells improve remyelination in demyelination model." *Mol Biotechnol* **56**(5): 387-93.
48. Odeh, H. M., C. Kleinguetl, et al. "Regulation of the proliferation and differentiation of Leydig stem cells in the adult testis." *Biol Reprod* **90**(6): 123.
49. Papagiannouli, F. and I. Lohmann "Stage-specific control of stem cell niche architecture in the Drosophila testis by the posterior Hox gene Abd-B." *Comput Struct Biotechnol J* **13**: 122-30.
50. Qian, Y., C. L. Ng, et al. "CSN maintains the germline cellular microenvironment and controls the level of stem cell genes via distinct CRLs in testes of Drosophila melanogaster." *Dev Biol* **398**(1): 68-79.
51. Ramm, S. A. and L. Schärer "The evolutionary ecology of testicular function: size isn't everything." *Biol Rev Camb Philos Soc* **89**(4): 874-88.
52. Sachs, C., B. D. Robinson, et al. "Evaluation of candidate spermatogonial markers ID4 and GPR125 in testes of adult human cadaveric organ donors." *Andrology* **2**(4): 607-14.
53. Sadri-Ardekani, H. and A. Atala "Testicular tissue cryopreservation and spermatogonial stem cell transplantation to restore fertility: from bench to bedside." *Stem Cell Res Ther* **5**(3): 68.
54. Sakurai, K., I. Talukdar, et al. "Kinome-wide functional analysis highlights the role of cytoskeletal remodeling in somatic cell reprogramming." *Cell Stem Cell* **14**(4): 523-34.
55. Shapiro-Kulnane, L., A. E. Smolko, et al. "Maintenance of Drosophila germline stem cell sexual identity in oogenesis and tumorigenesis." *Development* **142**(6): 1073-82.
56. Slaidina, M. and R. Lehmann "Translational control in germline stem cell development." *J Cell Biol* **207**(1): 13-21.
57. Tzung, K. W., R. Goto, et al. "Early depletion of primordial germ cells in zebrafish promotes testis formation." *Stem Cell Reports* **4**(1): 61-73.
58. van den Driesche, S., R. M. Sharpe, et al. "Regulation of the germ stem cell niche as the foundation for adult spermatogenesis: a role for miRNAs?" *Semin Cell Dev Biol* **29**: 76-83.
59. Voog, J., S. L. Sandall, et al. "Escargot restricts niche cell to stem cell conversion in the Drosophila testis." *Cell Rep* **7**(3): 722-34.
60. Vrooman, L. A., J. M. Oatley, et al. "Estrogenic exposure alters the spermatogonial stem cells in the developing testis, permanently reducing crossover levels in the adult." *PLoS Genet* **11**(1): e1004949.
61. Wikipedia. The free encyclopedia. <http://en.wikipedia.org>. 2015.
62. Xie, W., J. Sun, et al. "Construction and analysis of a protein-protein interaction network related to self-renewal of mouse spermatogonial stem cells." *Mol Biosyst* **11**(3): 835-43.

3/12/2015