

Spring 2026 – Systems Biology of Reproduction
Lecture Outline – Testis Systems Biology
Eric Nilsson – Biol 475/575
10:35-11:50 am, Tuesday & Thursday
February 24, 2026
Week 7

Testis Systems Biology

- Cell Biology of the Testis
 - Cell Types and Organization
 - Cell Associations

- Spermatogenesis
 - Stages and Cycle
 - Germ Cell Differentiation
 - Genes Involved

- Endocrinology of the Testis
 - Gonadotropins
 - Testosterone and Leydig Cell

- Cell-Cell Interactions
 - Types of Interactions
 - Sertoli-Germ Cell Interactions
 - Other Cellular Interactions

Required Reading

de Kretser, et al. (2018) Structure/Cells Overview. In: Encyclopedia of Reproduction (Second Edition). Volume 1, Pages 10-16

REFERENCES

- Byrne CJ, Keogh K, Kenny DA. Role of early life nutrition in regulating sexual development in bulls. *Animal*. 2023 May;17 Suppl 1:100802.
- Rajachandran S, Zhang X, Cao Q. Dissecting the spermatogonial stem cell niche using spatial transcriptomics. *Cell Rep*. 2023 Jul 25;42(7):112737.
- Zhang X, Cao Q, Rajachandran S, Grow EJ, Evans M, Chen H. Dissecting mammalian reproduction with spatial transcriptomics. *Hum Reprod Update*. 2023 Nov 2;29(6):794-810.

Haider S, Beristain AG. Human organoid systems in modeling reproductive tissue development, function, and disease. *Hum Reprod.* 2023 Aug 1;38(8):1449-1463.

Lu Y, Nagamori I, Kobayashi H, et al. ADAD2 functions in spermiogenesis and piRNA biogenesis in mice. *Andrology.* 2023 May;11(4):698-709.

Murat F, Mbengue N, Winge SB, et al. The molecular evolution of spermatogenesis across mammals. *Nature.* 2023 Jan;613(7943):308-316.

Sou IF, Hamer G, Tee W-W, et al. Cancer and meiotic gene expression: Two sides of the same coin? *Curr Top Dev Biol.* 2023;151:43-68.

Peart NJ, Johnson TA, Lee S, et al. The germ cell-specific RNA binding protein RBM46 is essential for spermatogonial differentiation in mice. *PLoS Genet.* 2022 Sep 21;18(9):e1010416.

Davila RA, Spiller C, Harkins D, et al. Deletion of NFIX results in defective progression through meiosis within the mouse testis. *Biol Reprod.* 2022 Jun 13;106(6):1191-1205.

Aydin Y, Yilnaz BO, Yildizbayrak N, et al. Evaluation of citrinin-induced toxic effects on mouse Sertoli cells. *Drug Chem Toxicol.* 2021 Nov;44(6):559-565.

Sou IF, Pryce RM, Tee W-W, McClurg UL. Meiosis initiation: a story of two sexes in all creatures great and small. *Biochem J.* 2021 Oct 29;478(20):3791-3805.

Mohammed SS, Mansour MF, Salem NA. Therapeutic Effect of Stem Cells on Male Infertility in a Rat Model: Histological, Molecular, Biochemical, and Functional Study. *Stem Cells Int.* 2021 Oct 25;2021:8450721.

Oliver E, Alves-Lopes JP, Femke Hartevelde F., et al. Self-organising human gonads generated by a Matrigel-based gradient system. *BMC Biol.* 2021 Sep 23;19(1):212.

Nagirnaja L, Mørup N, Nielsen JE, et al. Variant PNLDC1, Defective piRNA Processing, and Azoospermia. *N Engl J Med.* 2021 Aug 19;385(8):707-719.

Yamamuro T, Nakamura S, Yamano Y, et al. Rubicon prevents autophagic degradation of GATA4 to promote Sertoli cell function. *PLoS Genet.* 2021 Aug 5;17(8):e1009688.

Naemi S, Eidi A, Khanbabae R, et al. Differentiation and proliferation of spermatogonial stem cells using a three-dimensional decellularized testicular scaffold: a new method to study the testicular microenvironment in vitro. *Int Urol Nephrol.* 2021 Aug;53(8):1543-1550.

Shen Y-C, Shami AN, Moritz L, et al. TCF21 + mesenchymal cells contribute to testis somatic cell development, homeostasis, and regeneration in mice. *Nat Commun.* 2021 Jun 23;12(1):3876.

Zheng W, Zhang Y, Sun C, et al. A Multi-Omics Study of Human Testis and Epididymis. *Molecules.* 2021 Jun 2;26(11):3345.

Wang M, Yang Y, Cansever D, et al. Two populations of self-maintaining monocyte-independent macrophages exist in adult epididymis and testis. *Proc Natl Acad Sci U S A.* 2021 Jan 5;118(1):e2013686117.

Gewiss RL, Law NC, Helsel AR, Shelden EA, Griswold MD. Two distinct Sertoli cell states are regulated via germ cell crosstalk. *Biol Reprod.* 2021 Sep 7;ioab160.

Mori Y, Takashima S, Kanatsu-Shinohara M, et al. Cdc42 is required for male germline niche development in mice. *Cell Rep.* 2021 Aug 17;36(7):109550.

Hsu C-W, Chung B-C. Evolution, Expression, and Function of Gonadal Somatic Cell-Derived Factor. *Front Cell Dev Biol.* 2021 Jul 7;9:684352.

- Parker N, Laychur A, Sukwani M, et al. Spermatogonial Stem Cell Numbers Are Reduced by Transient Inhibition of GDNF Signaling but Restored by Self-Renewing Replication when Signaling Resumes. *Stem Cell Reports*. 2021 Mar 9;16(3):597-609.
- Wu S, Wang L, Tang EI, Wang J, Cheng CY. An In Vitro Assay to Monitor Sertoli Cell Blood-Testis Barrier (BTB) Integrity. *Methods Mol Biol*. 2021;2367:207-213.
- Yan R-G, Li B-Y, Yang Q-E. Function and transcriptomic dynamics of Sertoli cells during prospermatogonia development in mouse testis. *Reprod Biol*. 2020 Dec;20(4):525-535.
- Jorgez CJ, Seth A, Wilken N, et al. E2F1 regulates testicular descent and controls spermatogenesis by influencing WNT4 signaling. *Development*. 2021 Jan 13;148(1):dev191189.
- Wang Z-Y, Leushkin E, Liechti A, et al. Transcriptome and translome co-evolution in mammals. *Nature*. 2020 Dec;588(7839):642-647.
- Krausz C, Riera-Escamilla A, Moreno-Mendoza D, et al. Genetic dissection of spermatogenic arrest through exome analysis: clinical implications for the management of azoospermic men. *Genet Med*. 2020 Dec;22(12):1956-1966.
- Guo J, Sosa E, Chitiashvili T, et al. Single-cell analysis of the developing human testis reveals somatic niche cell specification and fetal germline stem cell establishment. *Cell Stem Cell*. 2021 Apr 1;28(4):764-778.e4.
- Kojima-Kita K, Kuramochi-Miyagawa S, Nakayama M, et al. MORC3, a novel MIWI2 association partner, as an epigenetic regulator of piRNA dependent transposon silencing in male germ cells. *Sci Rep*. 2021 Oct 14;11(1):20472.
- Du L, Chen W, Cheng Z, et al. Novel Gene Regulation in Normal and Abnormal Spermatogenesis. *Cells*. 2021 Mar 17;10(3):666.
- Liao J, HC, Luk ACS, et al. Transcriptomic and epigenomic profiling of young and aged spermatogonial stem cells reveals molecular targets regulating differentiation. *PLoS Genet*. 2021 Jul 8;17(7):e1009369.
- Oura S, Koyano T, Kodera C, et al. KCTD19 and its associated protein ZFP541 are independently essential for meiosis in male mice. *PLoS Genet*. 2021 May 7;17(5):e1009412.
- Capoano CA, Ortiz-Laquintana LA, Rodríguez-Casuriaga R, et al. SPATS1 (spermatogenesis-associated, serine-rich 1) is not essential for spermatogenesis and fertility in mouse. *PLoS One*. 2021 May 4;16(5):e0251028.
- Sung DC, Ahmad M, Cervantes CBL, et al. Mutations in non-muscle myosin 2A disrupt the actomyosin cytoskeleton in Sertoli cells and cause male infertility. *Dev Biol*. 2021 Feb;470:49-61.
- Strange DP, Jiyarom B, Sadri-Ardekani H, et al. Paracrine IFN Response Limits ZIKV Infection in Human Sertoli Cells. *Front Microbiol*. 2021 May 17;12:667146.
- Higuchi K, Matsumura T, Akiyama H, et al. Sertoli cell replacement in explanted mouse testis tissue supporting host spermatogenesis†. *Biol Reprod*. 2021 Oct 11;105(4):934-943.
- Shetty G, Mitchell JM, Lam TNA, et al. Postpubertal spermatogonial stem cell transplantation restores functional sperm production in rhesus monkeys irradiated before and after puberty. *Andrology*. 2021 Sep;9(5):1603-1616.
- Gillette R, Tiwary R, Voss JLP, Hewage SN, Richburg JH. Peritubular Macrophages Are Recruited to the Testis of Peripubertal Rats After Mono-(2-Ethylhexyl) Phthalate Exposure and Is Associated With Increases in the Numbers of Spermatogonia. *Toxicol Sci*. 2021 Aug 3;182(2):288-296.

Fleck D, Kenzler L, Mundt N, et al. ATP activation of peritubular cells drives testicular sperm transport. *Elife*. 2021 Jan 27;10:e62885.

Subash SK, Kumar PG. Spermatogonial stem cells: A story of self-renewal and differentiation. *Front Biosci (Landmark Ed)*. 2021 Jan 1;26:163-205.

Yan R-G, Li B-Y, Yang Q-E. Function and transcriptomic dynamics of Sertoli cells during prospermatogonia development in mouse testis. *Reprod Biol*. 2020 Dec;20(4):525-535.

Richer, Baert, Goossens (2019) In-vitro spermatogenesis through testis modelling: towards the generation of testicular organoids. *Andrology*. Epub ahead of print: 11 December 2019. <https://doi.org/10.1111/andr.12741>

Correia B, Sousa MI, Ramalho-Santos J. The mTOR Pathway in Reproduction: From Gonadal Function to Developmental Coordination. *Reproduction*. 2019 Nov 1. pii: REP-19-0057.R2. doi: 10.1530/REP-19-0057. [Epub ahead of print]

Brown JC. Control of human testis-specific gene expression. *PLoS One*. 2019 Sep 12;14(9):e0215184.

Liang J, Wang N, He J, Du J, Guo Y, Li L, Wu W, Yao C, Li Z, Kee K. Induction of Sertoli-like cells from human fibroblasts by NR5A1 and GATA4. *Elife*. 2019 Nov 11;8. pii: e48767. doi: 10.7554/eLife.48767.

Sakib S, Goldsmith T, Voigt A, Dobrinski I. Testicular organoids to study cell-cell interactions in the mammalian testis. *Andrology*. 2019 Jul 21. doi: 10.1111/andr.12680. [Epub ahead of print]

Yoshida S. Heterogeneous, dynamic, and stochastic nature of mammalian spermatogenic stem cells. *Curr Top Dev Biol*. 2019;135:245-285.

de Siqueira-Silva DH, da Silva Rodrigues M, Nóbrega RH. Testis structure, spermatogonial niche and Sertoli cell efficiency in Neotropical fish. *Gen Comp Endocrinol*. 2019 Mar 1;273:218-226.

Kubota H, Brinster RL. Spermatogonial stem cells. *Biol Reprod*. 2018 Jul 1;99(1):52-74

Gewiss R, Topping T, Griswold MD. Cycles, waves, and pulses: Retinoic acid and the organization of spermatogenesis. *Andrology*. 2019 Oct 31. doi: 10.1111/andr.12722. [Epub ahead of print]

Crisóstomo L, Alves MG, Gorga A, Sousa M, Riera MF, Galardo MN, Meroni SB, Oliveira PF. Molecular Mechanisms and Signaling Pathways Involved in the Nutritional Support of Spermatogenesis by Sertoli Cells. *Methods Mol Biol*. 2018;1748:129-155.

Sharma S, Wistuba J, Pock T, Schlatt S, Neuhaus N. Spermatogonial stem cells: updates from specification to clinical relevance. *Hum Reprod Update*. 2019 May 1;25(3):275-297.

Heinrich A, DeFalco T. Essential roles of interstitial cells in testicular development and function. *Andrology*. 2019 Aug 24. doi: 10.1111/andr.12703. [Epub ahead of print]

Yu K, Zhang Y, Zhang BL, Wu HY, Jiang WQ, Wang ST, Han DP, Liu YX, Lian ZX, Deng SL. In-vitro differentiation of early pig spermatogenic cells to haploid germ cells. *Mol Hum Reprod*. 2019 Sep 1;25(9):507-518.

Mäkelä JA, Hobbs RM. Molecular regulation of spermatogonial stem cell renewal and differentiation. *Reproduction*. 2019 Jun 1. pii: REP-18-0476.R2. doi: 10.1530/REP-18-0476. [Epub ahead of print]

Law NC, Oatley MJ, Oatley JM. Developmental kinetics and transcriptome dynamics of stem cell specification in the spermatogenic lineage. *Nat Commun*. 2019 Jun 26;10(1):2787.

Mohaqiq M, Movahedin M, Mazaheri Z, Amirjannati N. In vitro transplantation of spermatogonial stem cells isolated from human frozen-thawed testis tissue can induce

- spermatogenesis under 3-dimensional tissue culture conditions. *Biol Res.* 2019 Mar 27;52(1):16.
- Ziaepour S, Ahrabi B, Naserzadeh P, Aliaghaei A, et al. Effects of Sertoli Cell Transplantation on Spermatogenesis in Azoospermic Mice. *Cell Physiol Biochem.* 2019;52(3):421-434.
- Mäkelä JA, Koskenniemi JJ, Virtanen HE, Toppari J. Testis Development. *Endocr Rev.* 2019 Aug 1;40(4):857-905.
- Sosa E, Chen D, Rojas EJ, Hennebold JD, et al. Differentiation of primate primordial germ cell-like cells following transplantation into the adult gonadal niche. *Nat Commun.* 2018 Dec 17;9(1):5339.
- AbuMadighem A, Solomon R, Stepanovsky A, et al. Development of Spermatogenesis In Vitro in Three-Dimensional Culture from Spermatogonial Cells of Busulfan-Treated Immature Mice. *Int J Mol Sci.* 2018 Nov 29;19(12). pii: E3804. doi: 10.3390/ijms19123804.
- Guo J, Grow EJ, Mlcochova H, Maher GJ, Lindskog C, et al. The adult human testis transcriptional cell atlas. *Cell Res.* 2018 Dec;28(12):1141-1157.
- Chen N, Lin M, Liu N, Wang S, Xiao X. Methylmercury-induced testis damage is associated with activation of oxidative stress and germ cell autophagy. *J Inorg Biochem.* 2019 Jan;190:67-74.
- Gholami K, Pourmand G, Koruji M, Ashouri S, Abbasi M. Organ culture of seminiferous tubules using a modified soft agar culture system. *Stem Cell Res Ther.* 2018 Sep 26;9(1):249.
- Ribeiro MA, Estill MS, Fernandez GJ, Moraes LN, Krawetz SA, Scarano WR. Integrative transcriptome and microRNome analysis identifies dysregulated pathways in human Sertoli cells exposed to TCDD. *Toxicology.* 2018 Nov 1;409:112-118.
- Ramos-Treviño J, Bassol-Mayagoitia S, Hernández-Ibarra JA, Ruiz-Flores P, Nava-Hernández MP. Toxic Effect of Cadmium, Lead, and Arsenic on the Sertoli Cell: Mechanisms of Damage Involved. *DNA Cell Biol.* 2018 Jul;37(7):600-608.
- Bhattacharya I, Basu S, Pradhan BS, Sarkar H, Nagarajan P, Majumdar SS. Testosterone augments FSH signaling by upregulating the expression and activity of FSH-Receptor in Pubertal Primate Sertoli cells. *Mol Cell Endocrinol.* 2019 Feb 15;482:70-80.
- Griswold MD. 50 years of spermatogenesis: Sertoli cells and their interactions with germ cells. *Biol Reprod.* 2018 Jul 1;99(1):87-100.
- Zhou R, Wu J, Liu B, Jiang Y, Chen W, Li J, He Q, He Z. The roles and mechanisms of Leydig cells and myoid cells in regulating spermatogenesis. *Cell Mol Life Sci.* 2019 Jul;76(14):2681-2695.
- Shima Y, Miyabayashi K, Sato T, Suyama M, Ohkawa Y, Doi M, Okamura H, Suzuki K. Fetal Leydig cells dedifferentiate and serve as adult Leydig stem cells. *Development.* 2018 Dec 5;145(23).
- Chen P, Zirkin BR, Chen H. Leydig Cells in the Adult Testis: Characterization, Regulation and Potential Applications. *Endocr Rev.* 2019 Nov 1. pii: bnz013. doi: 10.1210/endrev/bnz013. [Epub ahead of print]
- Zirkin BR, Papadopoulos V. Leydig cells: formation, function, and regulation. *Biol Reprod.* 2018 Jul 1;99(1):101-111.
- Capel B. To Be or Not To Be a Testis. *Reproduction.* 2019 Jul 1. pii: REP-19-0151.R1. doi: 10.1530/REP-19-0151. [Epub ahead of print]
- Meroni SB, Galardo MN, Rindone G, Gorga A, Riera MF, Cigorraga SB. Molecular Mechanisms and Signaling Pathways Involved in Sertoli Cell Proliferation. *Front Endocrinol (Lausanne).* 2019 Apr 16;10:224.

- Gholami K, Pourmand G, Koruji M, Ashouri S, Abbasi M. Organ culture of seminiferous tubules using a modified soft agar culture system. *Stem Cell Res Ther.* 2018 Sep 26;9(1):249.
- Kutchy NA, Velho A, Menezes ESB, Jacobsen M, Thibaudeau G, Wills RW, Moura A, Kaya A, Perkins A, Memili E. (2017) Testis specific histone 2B is associated with sperm chromatin dynamics and bull fertility-a pilot study. *Reprod Biol Endocrinol.* 15(1):59.
- Ahn J, Park YJ, Chen P, Lee TJ, Jeon YJ, Croce CM, Suh Y, Hwang S, Kwon WS, Pang MG, Kim CH, Lee SS, Lee K. (2017) Comparative expression profiling of testis-enriched genes regulated during the development of spermatogonial cells. *PLoS One.* 17;12(4):e0175787.
- Alves-Lopes JP, Stukenborg JB. (2017) Testicular organoids: a new model to study the testicular microenvironment in vitro? *Hum Reprod Update.* 2017 Dec 21. doi: 10.1093/humupd/dmx036. [Epub ahead of print]
- Nakamura N, Merry GE, Inselman AL, Sloper DT, Del Valle PL, Sato T, Ogawa T, Hansen DK. (2017) Evaluation of Culture Time and Media in an In Vitro Testis Organ Culture System. *Birth Defects Res.* 109(7):465-474.
- Vicens A, Borziak K, Karr TL, Roldan ERS, Dorus S. (2017) Comparative Sperm Proteomics in Mouse Species with Divergent Mating Systems. *Mol Biol Evol.* 34(6):1403-1416.
- Ben Maamar M, Lesné L, Hennig K, Desdoits-Lethimonier C, Kilcoyne KR, Coiffec I, Rolland AD, Chevrier C, Kristensen DM, Lavoué V, Antignac JP, Le Bizec B, Dejucq-Rainsford N, Mitchell RT, Mazaud-Guittot S, Jégou B. (2017) Ibuprofen results in alterations of human fetal testis development. *Sci Rep.* 7:44184.
- Alikhani M, Mirzaei M, Sabbaghian M, Parsamatin P, Karamzadeh R, Adib S, Sodeifi N, Gilani MAS, Zabet-Moghaddam M, Parker L, Wu Y, Gupta V, Haynes PA, Gourabi H, Baharvand H, Salekdeh GH. (2017) Quantitative proteomic analysis of human testis reveals system-wide molecular and cellular pathways associated with non-obstructive azoospermia. *J Proteomics.* 162:141-154.
- Wen K, Yang L, Xiong T, Di C, Ma D, Wu M, Xue Z, Zhang X, Long L, Zhang W, Zhang J, Bi X, Dai J, Zhang Q, Lu ZJ, Gao G. (2016) Critical roles of long noncoding RNAs in *Drosophila* spermatogenesis. *Genome Res.* 26(9):1233-44.
- Miyata H, Castaneda JM, Fujihara Y, Yu Z, et al. (2016) Genome engineering uncovers 54 evolutionarily conserved and testis-enriched genes that are not required for male fertility in mice. *Proc Natl Acad Sci U S A.* 12;113(28):7704-10.
- Smith LB, O'Shaughnessy PJ, Rebouret D. (2015) Cell-specific ablation in the testis: what have we learned? *Andrology.* 3(6):1035-49
- Riaz MA, Stammner A, Borgers M, Konrad L. (2017) Clusterin signals via ApoER2/VLDLR and induces meiosis of male germ cells. *Am J Transl Res.* 9(3):1266-1276.
- Krausz C, Casamonti E. (2017) Spermatogenic failure and the Y chromosome. *Hum Genet.* 136(5):637-655.
- Potter SJ, DeFalco T. (2017) Role of the testis interstitial compartment in spermatogonial stem cell function. *Reproduction.* 153(4):R151-R162.
- Estill MS, Krawetz SA. (2016) The Epigenetic Consequences of Paternal Exposure to Environmental Contaminants and Reproductive Toxicants. *Curr Environ Health Rep.* 3(3):202-13.
- Stanton PG. (2016) Regulation of the blood-testis barrier. *Semin Cell Dev Biol.* 59:166-173.
- Navratilova P, Danks GB, Long A, Butcher S, Manak JR, Thompson EM. (2017) Sex-specific chromatin landscapes in an ultra-compact chordate genome. *Epigenetics Chromatin.* 17;10:3.

- Ueda J, Harada A, Urahama T, Machida S, et al. (2017) Testis-Specific Histone Variant H3t Gene Is Essential for Entry into Spermatogenesis. *Cell Rep.* 18(3):593-600.
- O'Donnell L, Stanton P, de Kretser DM. (2017) Endocrinology of the Male Reproductive System and Spermatogenesis. In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, Koch C, Korbonits M, McLachlan R, New M, Purnell J, Rebar R, Singer F, Vinik A, editors. *Source Endotext* [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-.2017 Jan 11.
- Nynca J, Dietrich MA, Adamek M, Steinhagen D, Bilińska B, Hejmej A, Ciereszko A. (2017) Purification, characterization and expression of transferrin from rainbow trout seminal plasma. *Comp Biochem Physiol B Biochem Mol Biol.* 208-209:38-46.
- Adolfi MC, Herpin A, Regensburger M, Sacquegno J, Waxman JS, Scharl M. (2016) Retinoic acid and meiosis induction in adult versus embryonic gonads of medaka. *Sci Rep.* 6:34281.
- She ZY, Yang WX. (2017) Sry and SoxE genes: How they participate in mammalian sex determination and gonadal development? *Semin Cell Dev Biol.* 63:13-22.
- Bittman EL. (2016) Timing in the Testis. *J Biol Rhythms.* 31(1):12-36.
- Li N, Tang EI, Cheng CY. (2016) Regulation of blood-testis barrier by actin binding proteins and protein kinases. *Reproduction.* 151(3):R29-41.
- Busada JT, Geyer CB. (2016) The Role of Retinoic Acid (RA) in Spermatogonial Differentiation. *Biol Reprod.* 94(1):10.
- Griswold MD. (2016) Spermatogenesis: The Commitment to Meiosis. *Physiol Rev.* 96(1):1-17.
- Young JC, Wakitani S, Loveland KL. (2015) TGF- β superfamily signaling in testis formation and early male germline development. *Semin Cell Dev Biol.* 45:94-103.
- Mruk DD, Cheng CY. (2015) The Mammalian Blood-Testis Barrier: Its Biology and Regulation. *Endocr Rev.* 36(5):564-91.
- Greenspan LJ, de Cuevas M, Matunis E. (2015) Genetics of Gonadal Stem Cell Renewal. *Annu Rev Cell Dev Biol.* 31:291-315.
- Chojnacka K, Mruk DD. (2015) The Src non-receptor tyrosine kinase paradigm: New insights into mammalian Sertoli cell biology. *Mol Cell Endocrinol.* 5;415:133-42.
- Tremblay JJ. (2015) Molecular regulation of steroidogenesis in endocrine Leydig cells. *Steroids.* 103:3-10.
- Lin YT, Capel B. (2015) Cell fate commitment during mammalian sex determination. *Curr Opin Genet Dev.* 32:144-52.
- Qian X, Mruk DD, Cheng YH, et al. (2014) Actin binding proteins, spermatid transport and spermiation. *Semin Cell Dev Biol.* 30:75-85.
- Kaur G, Thompson LA, Dufour JM. (2014) Sertoli cells--immunological sentinels of spermatogenesis. *Semin Cell Dev Biol.* 2014 Jun;30:36-44.
- O'Donnell L, O'Bryan MK. (2014) Microtubules and spermatogenesis. *Semin Cell Dev Biol.* 30:45-54.
- Smith LB. (2016) Nonclassical Testosterone Signaling: A New Pathway Controlling Spermatogenesis? *Biol Reprod.* 2016 Jan 6. pii: biolreprod.115.137950. [Epub ahead of print]
- Chapman KM, Medrano GA, Chaudhary J, Hamra FK. (2015) NRG1 and KITL Signal Downstream of Retinoic Acid in the Germline to Support Soma-Free Syncytial Growth of Differentiating Spermatogonia. *Cell Death Discov.* 2015;1. pii: 15018. Epub 2015 Oct 5.

- Odet F, Pan W, Bell TA, et al., (2015) The Founder Strains of the Collaborative Cross Express a Complex Combination of Advantageous and Deleterious Traits for Male Reproduction. *G3* (Bethesda). Oct 19;5(12):2671-83.
- Endo T, Romer KA, Anderson EL, et al., (2015) Periodic retinoic acid-STRA8 signaling intersects with periodic germ-cell competencies to regulate spermatogenesis. *Proc Natl Acad Sci U S A*. 112(18):E2347-56.
- Lindeman RE, Gearhart MD, Minkina A, et al. (2015) Sexual cell-fate reprogramming in the ovary by DMRT1. *Curr Biol*. 16;25(6):764-71.
- Kim B, Roy J, Shum WW, Da Silva N, Breton S. (2015) Role of testicular luminal factors on Basal cell elongation and proliferation in the mouse epididymis. *Biol Reprod*. 92(1):9.
- Haselman JT, Olmstead AW, Degitz SJ. (2015) Global gene expression during early differentiation of *Xenopus* (*Silurana*) *tropicalis* gonad tissues. *Gen Comp Endocrinol*. 1;214:103-13.
- Takehana Y, Matsuda M, Myosho T, et al. (2014) Co-option of Sox3 as the male-determining factor on the Y chromosome in the fish *Oryzias dancena*. *Nat Commun*. 20;5:4157.
- Liao HF¹, Chen WS², Chen YH³, et al., (2014) DNMT3L promotes quiescence in postnatal spermatogonial progenitor cells. *Development*. 141(12):2402-13.
- Puri P, Walker WH. (2016) The Regulation of Male Fertility by the PTPN11 Tyrosine Phosphatase. *Semin Cell Dev Biol*. pii: S1084-9521(16)30020-9. doi: 10.1016/j.semcdb.2016.01.020. [Epub ahead of print]
- Gao Y, Mruk DD, Cheng CY. (2015) Sertoli cells are the target of environmental toxicants in the testis - a mechanistic and therapeutic insight. *Expert Opin Ther Targets*. 19(8):1073-90.
- Beattie MC, Adekola L, Papadopoulos V, Chen H, Zirkin BR. (2015) Leydig cell aging and hypogonadism. *Exp Gerontol*. 68:87-91.
- Campos E, Stafford JM, Reinberg D. (2014) Epigenetic inheritance: histone bookmarks across generations. *Trends Cell Biol*. 24(11):664-74.
- Cheng CY. (2015) Toxicants target cell junctions in the testis: Insights from the indazole-carboxylic acid model. *Spermatogenesis*. 4(2):e981485.
- Murphy CJ, Richburg JH. (2015) Implications of Sertoli cell induced germ cell apoptosis to testicular pathology. *Spermatogenesis*. 4(2):e979110.
- Gungor-Ordueri NE, Mruk DD, Wan HT, et al., (2014) New insights into FAK function and regulation during spermatogenesis. *Histol Histopathol*. 29(8):977-89.
- Tremblay JJ. (2015) Molecular regulation of steroidogenesis in endocrine Leydig cells. *Steroids*. 103:3-10.
- Larney C, Bailey TL, Koopman P. (2014) Switching on sex: transcriptional regulation of the testis-determining gene *Sry*. *Development*. 141(11):2195-205.
- Smith LB, Walker WH. (2014) The regulation of spermatogenesis by androgens. *Semin Cell Dev Biol*. 30:2-13.
- Sarkar DK. (2016) Male germline transmits fetal alcohol epigenetic marks for multiple generations: a review. *Addict Biol*. 21(1):23-34.
- Crujeiras AB, Casanueva FF. (2015) Obesity and the reproductive system disorders: epigenetics as a potential bridge. *Hum Reprod Update*. 21(2):249-61.
- Thorup J, Nordenskjöld A, Hutson JM. (2014) Genetic and environmental origins of hypospadias. *Curr Opin Endocrinol Diabetes Obes*. 21(3):227-32.

- Moreno-Ruiz P, Arluzea J, Silván U, et al., (2016) Testis peritubular myoid cells increase their motility and express matrix-metalloproteinase 9 (MMP-9) after interaction with embryonal carcinoma cells. *Andrology*. 4(1):111-20.
- Luo LF, Hou CC, Yang WX. (2016) Small non-coding RNAs and their associated proteins in spermatogenesis. *Gene*. 10;578(2):141-57.
- Browne JA, Yang R1 Leir SH, Eggener SE, Harris A. (2016) Expression profiles of human epididymis epithelial cells reveal the functional diversity of caput, corpus and cauda regions. *Mol Hum Reprod*. 22(2):69-82.
- McClelland KS, Bell K, Larney C, et al., (2015) Purification and Transcriptomic Analysis of Mouse Fetal Leydig Cells Reveals Candidate Genes for Specification of Gonadal Steroidogenic Cells. *Biol Reprod*. 92(6):145.
- Urriola-Muñoz P, Lagos-Cabré R, Moreno RD. (2014) A mechanism of male germ cell apoptosis induced by bisphenol-A and nonylphenol involving ADAM17 and p38 MAPK activation. *PLoS One*. 9(12):e113793.
- Rebourcet D, O'Shaughnessy PJ, Monteiro A, et al., (2014) Sertoli cells maintain Leydig cell number and peritubular myoid cell activity in the adult mouse testis. *PLoS One*. 9(8):e105687.
- Carney CM, Muszynski JL, Strotman LN, et al. (2014) Cellular microenvironment dictates androgen production by murine fetal Leydig cells in primary culture. *Biol Reprod*. 91(4):85.
- Chalmel F, Com E, Lavigne R, et al., (2014) An integrative omics strategy to assess the germ cell secretome and to decipher sertoli-germ cell crosstalk in the Mammalian testis. *PLoS One*. 9(8):e104418.
- Rotgers E, Rivero-Müller A, Nurmio M, et al., (2014) Retinoblastoma protein (RB) interacts with E2F3 to control terminal differentiation of Sertoli cells. *Cell Death Dis*. 5;5:e1274.
- Chapman KM1, Medrano GA1, Chaudhary J1, Hamra FK2. (2015) NRG1 and KITL Signal Downstream of Retinoic Acid in the Germline to Support Soma-Free Syncytial Growth of Differentiating Spermatogonia. *Cell Death Discov*. 2015;1. pii: 15018. Epub 2015 Oct 5.
- Kläver R, Sánchez V, Damm OS, et al., (2015) Direct but no transgenerational effects of decitabine and vorinostat on male fertility. *PLoS One*. 18;10(2):e0117839.
- Hirayanagi Y, Qu N, Hirai S, et al., (2015) Busulfan pretreatment for transplantation of rat spermatogonia differentially affects immune and reproductive systems in male recipient mice. *Anat Sci Int*. 90(4):264-74.
- Ramm SA, Schärer L, Ehmcke J, Wistuba J. (2014) Sperm competition and the evolution of spermatogenesis. *Mol Hum Reprod*. 20(12):1169-79.
- Gibbs GM, Lo JC, Nixon B, et al. (2010) Glioma pathogenesis-related 1-like 1 is testis enriched, dynamically modified, and redistributed during male germ cell maturation and has a potential role in sperm-oocyte binding. *Endocrinology*. 151(5):2331-42.
- Reynard LN, Cocquet J, Burgoyne PS. (2009) The multi-copy mouse gene *Sycp3*-like Y-linked (*Sly*) encodes an abundant spermatid protein that interacts with a histone acetyltransferase and an acrosomal protein. *Biol Reprod*. 81(2):250-7.
- Majumdar SS, Bhattacharya I. (2013) Genomic and post-genomic leads toward regulation of spermatogenesis. *Prog Biophys Mol Biol*. 113(3):409-22.
- Manna PR, Cohen-Tannoudji J, Counis R, et al. (2013) Mechanisms of action of hormone-sensitive lipase in mouse Leydig cells: its role in the regulation of the steroidogenic acute regulatory protein. *J Biol Chem*. 22;288(12):8505-18.

- Sugimoto R, Nabeshima Y, Yoshida S. (2012) Retinoic acid metabolism links the periodical differentiation of germ cells with the cycle of Sertoli cells in mouse seminiferous epithelium. *Mech Dev.* 128(11-12):610-24.
- Sinden D, Badgett M, Fry J, et al (2012) Jak-STAT regulation of cyst stem cell development in the *Drosophila* testis. *Dev Biol.* 1;372(1):5-16.
- Sharma A, Singh P. (2009) Detection of transgenerational spermatogenic inheritance of adult male acquired CNS gene expression characteristics using a *Drosophila* systems model. *PLoS One.* 2;4(6):e5763.
- Xia Y, Schneyer AL. (2009) The biology of activin: recent advances in structure, regulation and function. *J Endocrinol.* 202(1):1-12.
- Desai SS, Roy BS, Mahale SD. (2013) Mutations and polymorphisms in FSH receptor: functional implications in human reproduction. *Reproduction.* 23;146(6):R235-48.
- Guerrero-Bosagna C, Savenkova M, Haque MM, Nilsson E, Skinner MK. (2013) Environmentally induced epigenetic transgenerational inheritance of altered Sertoli cell transcriptome and epigenome: molecular etiology of male infertility. *PLoS One.* 8(3):e59922.
- Su W, Liu X. (2013) RAB13 regulates Sertoli cell permeability barrier dynamics through protein kinase A. *J Mol Endocrinol.* 12;50(3):305-18.
- Li N, Wang T, Han D. (2012) Structural, cellular and molecular aspects of immune privilege in the testis. *Front Immunol.* 11;3:152.
- Oatley JM, Brinster RL. (2012) The germline stem cell niche unit in mammalian testes. *Physiol Rev.* 92(2):577-95.
- Itman C, Wong C, Whiley PA, Fernando D, Loveland KL. (2011) TGF β superfamily signaling regulators are differentially expressed in the developing and adult mouse testis. *Spermatogenesis.* 1(1):63-72.
- Pelletier RM. (2011) The blood-testis barrier: the junctional permeability, the proteins and the lipids. *Prog Histochem Cytochem.* 46(2):49-127.
- Kopera IA, Bilinska B, Cheng CY, Mruk DD. (2010) Sertoli-germ cell junctions in the testis: a review of recent data. *Philos Trans R Soc Lond B Biol Sci.* 27;365(1546):1593-605.
- Mithraprabhu S, Mendis S, Meachem SJ, et al. (2010) Activin bioactivity affects germ cell differentiation in the postnatal mouse testis in vivo. *Biol Reprod.* 82(5):980-90.
- Uhlenhaut NH, Jakob S, Anlag K, et al. (2009) Somatic sex reprogramming of adult ovaries to testes by FOXL2 ablation. *Cell.* 11;139(6):1130-42.
- Hu Z, Dandekar D, O'Shaughnessy PJ, et al. (2010) Androgen-induced RhoX homeobox genes modulate the expression of AR-regulated genes. *Mol Endocrinol.* 24(1):60-75.
- Welsh M, Saunders PT, Atanassova N, et al. (2009) Androgen action via testicular peritubular myoid cells is essential for male fertility. *FASEB J.* 23(12):4218-30.
- Walker WH. (2009) Molecular mechanisms of testosterone action in spermatogenesis. *Steroids.* 74(7):602-7.
- Miyabayashi K, Katoh-Fukui Y, Ogawa H, et al. (2013) Aristaless related homeobox gene, Arx, is implicated in mouse fetal Leydig cell differentiation possibly through expressing in the progenitor cells. *PLoS One.* 28;8(6):e68050.
- Beattie MC, Chen H, Fan J, et al. (2013) Aging and luteinizing hormone effects on reactive oxygen species production and DNA damage in rat Leydig cells. *Biol Reprod.* 18;88(4):100, 1-7.
- Shima Y, Miyabayashi K, Haraguchi S, et al. (2013) Contribution of Leydig and Sertoli cells to testosterone production in mouse fetal testes. *Mol Endocrinol.* 27(1):63-73.

- Zirkin BR, Tenover JL. (2012) Aging and declining testosterone: past, present, and hopes for the future. *J Androl.* 33(6):1111-8.
- Stanley E, Lin CY, Jin S, et al. (2012) Identification, proliferation, and differentiation of adult Leydig stem cells. *Endocrinology.* 153(10):5002-10.
- Díez-Torre A, Silván U, Moreno P, et al. (2011) Peritubular myoid cell-derived factors and its potential role in the progression of testicular germ cell tumours. *Int J Androl.* 34(4 Pt 2):e252-64; discussion e264-5.
- DeFalco T, Takahashi S, Capel B. (2011) Two distinct origins for Leydig cell progenitors in the fetal testis. *Dev Biol.* 1;352(1):14-26.
- D'Cruz SC, Vaithinathan S, Jubendradass R, Mathur PP. (2010) Effects of plants and plant products on the testis. *Asian J Androl.* 12(4):468-79.
- Zirkin BR. (2010) Where do adult Leydig cells come from? *Biol Reprod.* 82(6):1019-20.
- Lie PP, Mruk DD, Mok KW, et al. (2012) Focal adhesion kinase-Tyr407 and -Tyr397 exhibit antagonistic effects on blood-testis barrier dynamics in the rat. *Proc Natl Acad Sci U S A.* 31;109(31):12562-7.
- Gatta V, Raicu F, Ferlin A, et al. (2010) Testis transcriptome analysis in male infertility: new insight on the pathogenesis of oligo-azoospermia in cases with and without AZFc microdeletion. *BMC Genomics.* 24;11:401.
- Griswold MD. (2012) Making male gametes in culture. *Proc Natl Acad Sci U S A.* 16;109(42):16762-3.
- Meng J, Greenlee AR, Taub CJ, Braun RE. (2011) Sertoli cell-specific deletion of the androgen receptor compromises testicular immune privilege in mice. *Biol Reprod.* 85(2):254-60.
- Kopera IA, Bilinska B, Cheng CY, Mruk DD. (2010) Sertoli-germ cell junctions in the testis: a review of recent data. *Philos Trans R Soc Lond B Biol Sci.* 27;365(1546):1593-605.

Structure/Cells Overview

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Development of Testes

The testes are located in the scrotum since sperm production requires a temperature that is cooler than that of the abdomen (de Kretser, 2016). They develop in the embryo just distal to the kidneys and descend into the scrotum shortly after birth through the inguinal canal. This canal, found on both sides in the region of the groin, is formed by the attachments of one of the muscles of the abdominal wall. The canal extends downwards and medially in the groin and links the abdominal cavity with the scrotum (de Kretser, 2016; de Kretser et al., 1982; Clermont and Huckins, 1961; Roosen-Runge and Holstein, 1978; Hutson et al., 1990).

Descent of the Testes

In some males, the inguinal canal does not close and the testes may retract from the scrotum into the abdominal cavity for brief periods. The descent of the testis is important because the temperature of the scrotum is lower than the intra-abdominal temperature and the germ cells require a lower temperature for their survival (Hutson et al., 1992). In some males, the inguinal canal which normally closes after the testes descend, remains patent and the testes may retract for varying periods causing damage to the germ cells because of the higher intra-abdominal temperature. Descent of the testis begins in the fetus at about 28 weeks of gestation and should be complete by birth and it is controlled by a Leydig cell secreted protein called insulin-like protein 3 which is a member of the insulin-like protein super family (de Kretser et al., 1982; Bowles and Koopman, 2007).

Failure of the testes to descend should be diagnosed as soon possible as spermatogenic damage and infertility may result. Surgery can be undertaken to close off the inguinal canal so that the testes are permanently located in the scrotum. The testes are ovoid in shape and in adults, their volume ranges from 15 to 35 mL. At birth they are about 1–3 mL in volume grow rapidly during pubertal development. The availability of an orchidometer, a range of spheres from 1 to 3 mL in progressively increasing volumes to 35 mL, is every helpful in determining testicular size (Fig. 1).



Fig. 1 *Orchidometer*. This set of models of differing testicular size helps the physician in assessing the size of the testes in patients with delayed puberty, infertility or potential testicular tumors.

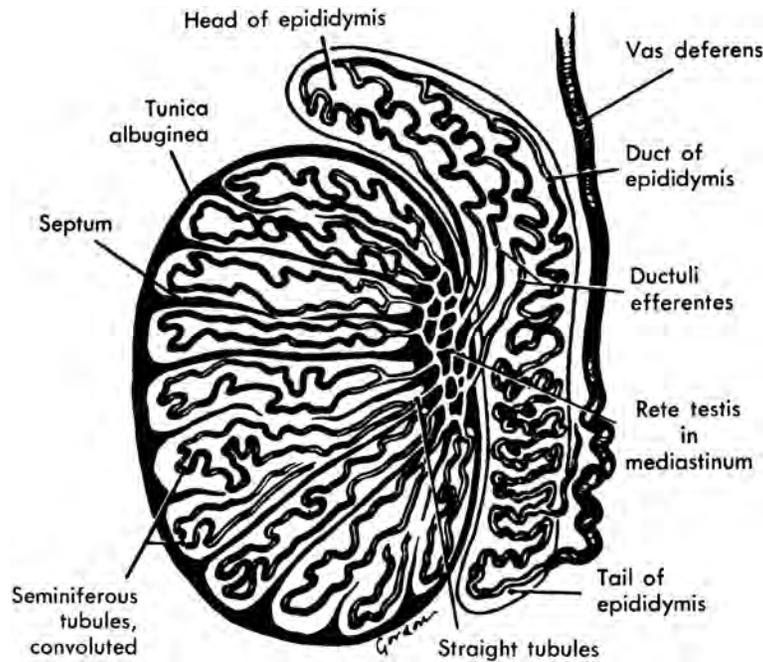


Fig. 2 The anatomical features of the testis, epididymis, vas deferens and the pampiniform plexus of veins, that surround the testicular artery, represent the venous drainage of the testis and epididymis.

The testes are covered by thick fibrous tissue that forms the tunica albuginea and should be smooth on their anterior and lateral surfaces. These surfaces are also covered by a serous membrane called the tunica vaginalis. Posteriorly, in the region under the epididymis, the tunica albuginea is thickened and projects into the parenchyma of the testes to form the mediastinum of the testis. A series of tubules traverse the mediastinum of the testis to link the seminiferous tubules to the efferent ducts that form the head of the epididymis (Roosen-Runge and Holstein, 1978) (Fig. 2).

In prepubertal boys, the germ cells in the testes are called gonocytes which are centrally placed in the seminiferous cords, that are the precursors of the seminiferous tubules. The other cellular components of the cords are the immature Sertoli cells that extend from the basement membrane of the tubule to the lumen of the seminiferous tubules. As development proceeds, the gonocytes move to the periphery of the cords to lie on the basement membrane of the cords and commence dividing by mitosis to give rise to the spermatogonial stem cells a process that requires retinoic acid and Oct 4 (de Rooij and Russell, 2000; de Rooij and Grootegeed, 1998; Dann et al., 2008; Bowles and Koopman, 2007). Continuation of spermatogonial mitosis requires the action of Foxo1 and the spermatogonial stem cells have the capacity for pluripotency, a characteristic marker of stem cells in many tissues (de Rooij and Russell, 2000; de Rooij, 2001; Goertz et al., 2011). When this occurs at the time of puberty, the basally placed Sertoli cells form specialized tight junctions just central to the gonocytes thereby preventing inter-cellular transport of substances and creating a blood–testis barrier (Dann et al., 2008). External to the basement membrane of the seminiferous tubules, there is a layer of myofibroblasts that can contract and increase the intra-tubular pressure. This facilitates the movement of sperm and the fluid produced by the Sertoli cells in to the rete testis (Simoni et al., 1999).

Functions

The testes have three functions, the production of sperm, the secretion of the steroid hormone, testosterone, after puberty and the production and secretion of protein hormones inhibin, activin, and follistatin. In addition, insulin and IGF1 are important in the control of Sertoli cell proliferation (Pitetti et al., 2013). Testosterone is synthesized and secreted by the Leydig cells of the testis that lie close to blood vessels found in the inter-tubular region of the testis. The Leydig cells have the characteristics of steroid secreting cells, namely a well developed smooth endoplasmic reticulum and mitochondria which have tubular cristae unlike “conventional” mitochondria in which the inner mitochondrial membrane forms “plate-like” cristae (de Kretser, 1967).

There are also lymphatic vessels in the inter-tubular compartment of the testes and these join abdominal lymphatics that also transport testosterone into the chest where they join the thoracic duct, the common duct of all lymphatic vessels in the body. The thoracic duct joins the venous system at the junction of the left subclavian vein and the left internal jugular vein (Stanton, 2016) (Fig. 2).

Spermatogenesis

The testes produce sperm in tubules termed seminiferous tubules by a process called spermatogenesis. These tubules are composed of germ cells and the supporting network of Sertoli cells. The tubules are surrounded by a layer of basement membrane, external to which lies a plate-like layer of peritubular cells which are contractile effectively “squeezing” the seminiferous tubules (Holstein et al., 1996; Simoni et al., 1999). Thereby they assist in moving sperm, released into the lumen of the seminiferous tubules, toward an irregular network of tubules located at the posterior and superior pole of the testis called the rete testis.

The rete testis is connected with the duct of the epididymis, a coiled tube that lies at the posterior aspect of the testis and is divided into the head, body and tail, the latter continuing as the vas deferens (Johnston and Whillis, 1954). In the epididymis, the sperm, which are still not motile, are moved by muscular contractions from the head to the tail of the epididymis. They acquire mobility as they pass through the epididymis to enter the vas deferens (Baker, 1989). The latter delivers the sperm during ejaculation to enter the prostatic urethra and pass through the penile urethra.

The cellular components in the seminiferous tubules are the germ cells that are the precursors of sperm and also the Sertoli cells. The latter are named after the person who first described them and they are a critical component of the seminiferous epithelium. They extend from the basement membrane of the tubule to the lumen and send projections between the surrounding germ cells not unlike the branches of a tree from the trunk. These projections contain microfilaments that provide a structural framework for the epithelium given that the germ cell components migrate from the basally placed spermatogonia to the centrally placed spermatids and their final product, the spermatozoa (Fig. 3).

In prepubertal boys, the germ cells in the testes are called gonocytes and they are centrally placed in the seminiferous cords that comprise the testis (Clermont and Huckins, 1961; de Rooij and Grootegoed, 1998; de Rooij and Russell, 2000). The other cells comprising the cords are the immature Sertoli cells that extend from the basement membrane of the cords surrounding the gonocytes. At the commencement of puberty, the gonocytes move to the periphery of the cords and the Sertoli cells form specialized cell junctions central to the gonocytes which will progress to give rise to the population of spermatogonia, the precursors to the subsequent stages of spermatogenesis (Johnston and Whillis, 1954). These changes, under the influence of the pubertal increase in FSH, act through Foxo1 and Oct 4 (Dann et al., 2008).

Where adjacent Sertoli cell projections meet basally, they form specialized tight cell junctions that prevent inter-cellular transport creating a blood–testis barrier (Dym and Fawcett, 1970; Russell, 1977). These tight junctions are placed at such a position in the seminiferous epithelium that only the spermatogonia are in contact with the basement membrane of the seminiferous tubules. All other germ cells lie central to the blood–testis barrier and are thus dependent on the Sertoli cells for transport of materials for optimal germ cell function and can be considered to “nurse” germ cells central to these inter-Sertoli cell junctions.

The inter-Sertoli cell junctions must open centrally to enable the progeny of spermatogonia, the primary spermatocytes, to leave the basal compartment and enter the luminal compartment. The inter-Sertoli cell junctions reform basally below the primary spermatocytes that now lie within adluminal compartment of the seminiferous tubule (Stanton, 2016).

Studies have shown that the number of Sertoli cells can affect the magnitude of sperm production. One of the important factors that controls Sertoli cell numbers is activin A which stimulates proliferation and inhibits differentiation of Sertoli cells (Baker, 1989; Kreuger et al., 1974). Increasing systemic levels of activin A using an adeno-associated virus expressing activin A (Russell, 1977) stimulated proliferation and prevented differentiation of Sertoli cells in mice. This was associated with disruption of the blood testis barrier formed by the inter-Sertoli cell junctions and resulted in a 23.5% decrease in testis weight due to diminished spermatogenesis linked to disordered Sertoli cell function. The latter was associated with increase in markers of juvenile Sertoli cells and a decrease in claudin-11, a marker of mature Sertoli cells. These data are consistent with studies of the levels of activin A in mice during normal post-natal development which established that activin A levels are elevated at birth but decline rapidly after day 4 postpartum (Meehan et al., 2000).

Other studies using treatment with FSH or thyroxine, a hormone secreted by the thyroid gland, can enhance Sertoli cell proliferation and thus increase sperm output.

In part, the action of FSH on spermatogenesis is exerted directly via spermatogonia which are the only germ cells that have FSH receptors (Simoni et al., 1999). Germ cells also do not have androgen receptors and thus the requirement of testosterone for successful spermatogenesis is dependent on the presence of androgen receptors on the Sertoli cells.

Since in the human, testicular sperm production continues from puberty throughout life, there is clearly a need for a population of stem cells to produce the precursor cells that develop into sperm. The cells forming this stem cell population are the spermatogonia that undergo several mitotic divisions and have 46 chromosomes as do all other cells in organs throughout the body (Amory et al., 2011). They develop from the gonocyte population found in the testes of prepubertal boys. The gonocytes are initially placed in the centre of the seminiferous cords and migrate to lie between the precursors of the Sertoli cells and, as with the gonocytes, the spermatogonia are basally placed in contact with the basement membrane of the seminiferous tubules. These cells undergo several stages of development and are designated by their cytological features before commencing meiosis.

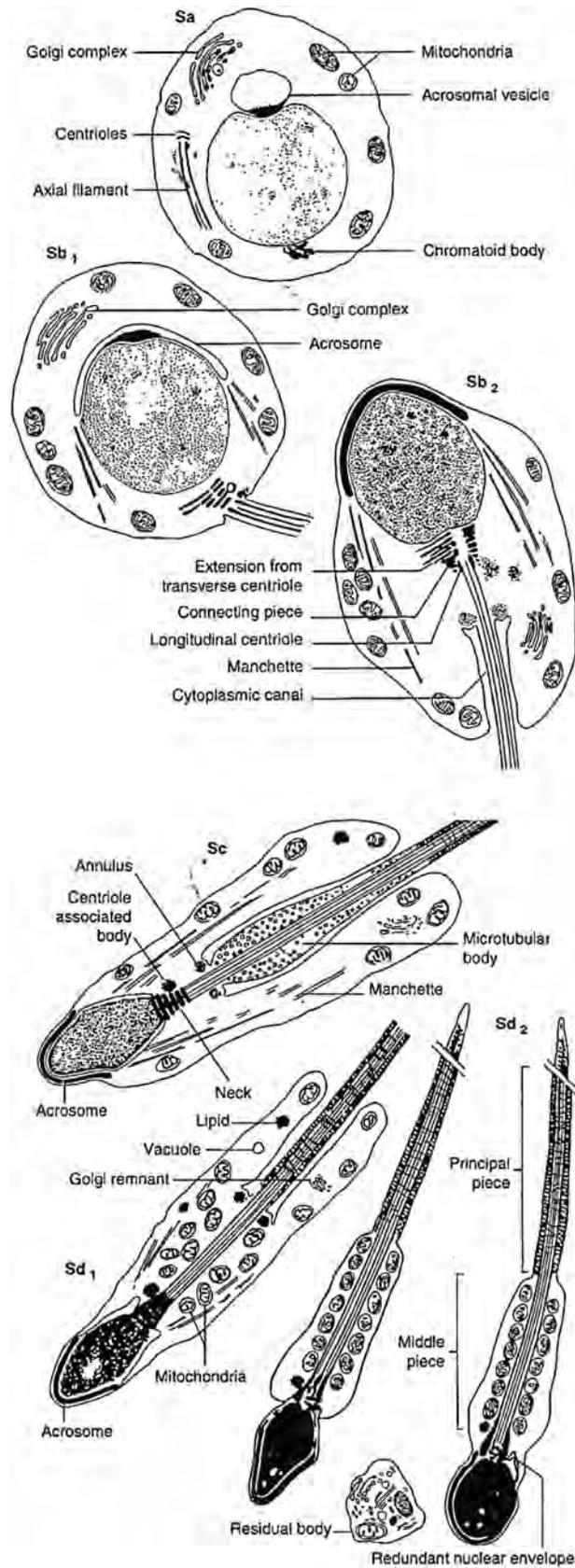


Fig. 3 The efferent ducts draining from the rete testis to form the head of the epididymis is illustrated together with the distal regions of the epididymis termed the body and tail.

The spermatogonia lose their contact with the basement membrane of the tubule when they begin another process of cell division called meiosis by which the chromosome numbers in these cells are reduced from 46 to 23. These cells are called primary spermatocytes.

During meiosis, the homologous chromosomes, derived originally from the fertilization of the egg, one provided by the mother, and the other member of the pair, provided by the father via his sperm, pair and replicate their DNA (deoxyribonucleic acid). The primary spermatocytes thus have nuclear features which enable the identification of the stages as the cells undergo the first meiotic division during which, one of each homologous chromosome pair, moves to the opposite pole of the cell. These cell types are named by the stage of meiosis that they have reached and can be identified by the chromatin pattern in their nucleus associated with the chromosome replication. Leptotene, zygotene, pachytene, diplotene, and diakinesis stages can be identified and, unlike cell division in somatic cells, these germ cells remain connected by intercellular bridges that link the cytoplasm of these cells. These bridges enable the development of the “chains” of germ cells and remain in place in the primary and secondary spermatocyte populations. This process requires the involvement of retinoic acid and androgens to proceed to completion. (Amory et al., 2011).

The completion the first meiotic division gives rise to cells called secondary spermatocytes that have half the number of chromosomes, 23, termed the haploid number, in contrast to their diploid precursor which had 46. The secondary spermatocytes then divide by mitosis to give rise to a further population of cells called round spermatids that are still connected by the cytoplasmic bridges.

Spermiogenesis

The round spermatids do not divide further but are transformed by a complex series of changes into a sperm, the process being called spermiogenesis (de Kretser, 1969). The basic changes in the developing spermatids during spermiogenesis are common to many mammalian species but the resulting sperm vary in their morphology especially in the shape of the head of the resulting sperm. The structure of the sperm tail however has many features in common across species. In the round spermatids the nucleus, which is centrally placed in the cell, is “capped” at one pole by a series of vesicles from the Golgi complex that coalesce to form a “cap” that is called the acrosome and is applied to that part of the nucleus closest to the acrosome. The acrosome covers approximately 30%–50% of the nuclear surface.

The nucleus, in the region of the acrosome, comes into close apposition with the cell membrane but remains separated from the nucleus by the acrosome.

Subsequently, as spermatid development continues, the nuclear chromatin undergoes a progressive condensation forming electron dense granules associated with stabilization of the DNA (Sassone-Corsi, 2002). That process involves the replacement of lysine-rich histones with transitional proteins, subsequently replaced by arginine-rich proteins called protamines. The nuclear chromatin granules condense as spermiogenesis progresses and it becomes more difficult to identify individual granules (Fig. 4).

At the pole of the nucleus opposite to the acrosome, a pair of centrioles, that participate in the development of the flagellum, lodge in a small fossa or indentation that still lies external to the nuclear membrane. This whole complex is called the connecting piece. The centriole closest to the nucleus, called the proximal centriole, lies at right angles to the plane of the distal centriole which gives rise to the core of the sperm tail called the axoneme. The axoneme is composed of a core of microtubules which forms the basis of the sperm tail sometimes called the axial filament (Fawcett, 1975).

The axial filament comprises nine pairs of doublet microtubules which surround two centrally placed single microtubules, a structure that is identical to the structure of cilia which also exhibit motility similar to the sperm tail.

A second set of nine outer dense fibers surround the axial filament distal to a dense ring termed the annulus. The annulus marks the distal end of the mid-piece and its mitochondrial sheath and defines the commencement of the fibrous sheath. The annulus marks the commencement of the region of the sperm tail called the principal-piece and the axonemal core, distal to the termination of the fibrous sheath, is termed the end-piece.

The final step, before sperm are released from the epithelium by a process called spermiation, is a movement of mitochondria, that up to this point have been distributed around the periphery of the spermatid cell membrane, to surround the mid-piece to form a “mitochondrial sheath” distal to the connecting-piece and ending at the annulus.

Spermiation, involves the release of sperm from the seminiferous epithelium. At this stage, the cytoplasm of the spermatid has migrated to a caudal position around the tail. Projections of Sertoli cells invaginate this caudal cytoplasmic collection to “literally” pull the residual cytoplasm off the spermatid, thereby releasing it into the lumen of the seminiferous tubule.

The residual bodies within the Sertoli cells, that contain the “unwanted” cellular components of the spermatids, are moved toward the base of the Sertoli cells and progressively “digested” by lysosomes. There is some data to suggest that these cellular components signal to the Sertoli cell that a “generation” of sperm have been released from that region of the epithelium.

The spermatozoa, that are released from the Sertoli cells are still immotile. They, together with fluid secreted by the Sertoli cells into the lumen of the seminiferous tubules, are moved toward the rete testis by the contractions of the peritubular myoid cells and enter the epididymis.

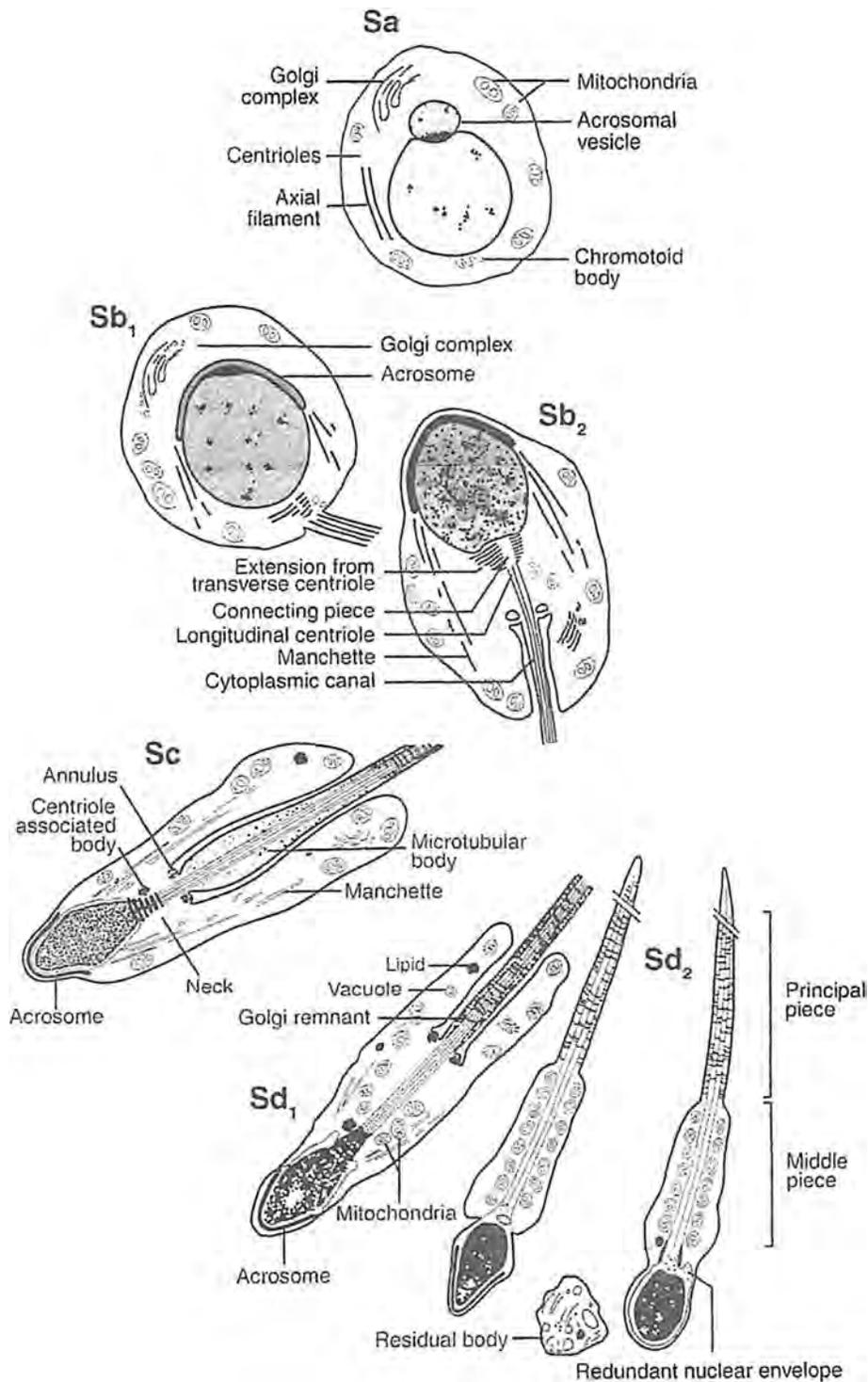


Fig. 4 The specific stages of spermiogenesis, the process by which a round spermatid (Sa) is transformed into a spermatozoon (Sd₂) are illustrated. Reproduced with permission from de Kretser, D.M. (1969). Ultrastructural features of human spermiogenesis. *Zeitschrift für Zellforschung* **98**, 477–505.

References

Amory, J. K., Muller, C. H., Shimshoni, J. A., et al. (2011). Suppression of spermatogenesis by bischloroacetyl diamines is mediated by the inhibition of testicular retinoic acid biosynthesis. *Journal of Andrology*, *32*, 111–119.

Baker, H. W. G. (1989). Clinical evaluation and management of testicular disorders in the adult. In H. G. Burger, & D. M. de Kretser (Eds.), *The testis* (2nd ed., pp. 419–440). New York: Raven Press.

- Bowles, J., & Koopman, P. (2007). Retinoic acid, meiosis and germ cell fate in mammals. *Development*, *134*, 3401–3411.
- Clermont, Y., & Huckins, C. (1961). Microscopic anatomy of the sex cords and seminiferous tubules in growing and adult male albino rats. *The American Journal of Anatomy*, *108*, 79–97.
- Dann, C. T., Alvarado, A. L., Molyneux, L. A., Denard, B. S., Garbers, D. L., & Forteus, M. H. (2008). Spermatogonial stem cell self-renewal requires OCT4, a factor down regulated during retinoic acid-induced differentiation. *Stem Cells*, *26*, 2928–2937.
- Dym, M., & Fawcett, D. W. (1970). The blood–testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium. *Biology of Reproduction*, *3*, 308–326.
- Fawcett, D. W. (1975). The mammalian spermatozoon. *Developmental Biology*, *44*, 394–436.
- Goertz, M. J., Wu, Z., Gallardo, T. D., Hamra, F. K., & Castrillon, D. H. (2011). Foxo1 is required in mouse spermatogonial stem cells for their maintenance and the initiation of spermatogenesis. *The Journal of Clinical Investigation*, *121*, 3456–3466.
- Holstein, A. F., Maekawa, M., Nagano, T., et al. (1996). Myofibroblasts in the lamina propria of human seminiferous tubules are dynamic structures of heterogeneous phenotype. *Archives of Histology and Cytology*, *59*, 109–125.
- Hutson, J. M., Williams, M. P. L., Fallat, M. E., & Attah, A. (1990). Testicular descent, new insights into its hormonal control. *Developmental and Reproductive Biology*, *12*, 1–56.
- Hutson, J. M., Baker, M. L., Griffiths, A. L., et al. (1992). Endocrine and morphological perspectives in testicular descent. *Reproductive Medicine Review*, *1*, 165–177.
- Johnston, T. B., & Whillis, J. (1954). The male genital organs. In *Gray's Anatomy* (31st edn., pp. 1462–1473). London: Longmans Green & Co.
- de Kretser, D. M. (1967). Changes in the fine structure of the human testicular interstitial cells after treatment with human gonadotrophin. *Zeitschrift für Zellforschung*, *83*, 344–358.
- de Kretser, D. M. (1969). Ultrastructural features of human spermiogenesis. *Zeitschrift für Zellforschung*, *98*, 477–505.
- de Kretser, D. M. (2016). *Endocrinology adult and pediatric* (7th edn., vol. 2). Philadelphia: Elsevier Saunders.
- de Kretser, D. M., Temple-Smith, P. D., & Kerr, J. B. (1982). Anatomical and functional aspects of the male reproductive organs. In K. Bandhauer, & J. Frick (Eds.), *Handbook of urology* (vol. 16, pp. 1–131). Berlin: Springer Verlag.
- Kreuger, P. M., Hodgen, G. D., & Sherins, R. J. (1974). New evidence for the role of the Sertoli cell and spermatogonia in feedback control of FSH secretion in male rats. *Endocrinology*, *95*, 955–962.
- Meehan, T., Schlatt, S., O'Bryan, M. K., de Kretser, D. M., & Loveland, K. L. (2000). Regulation of germ cell and Sertoli cell development by activin, follistatin and FSH. *Developmental Biology*, *220*, 225–237.
- Pitetti, J. L., Calvel, P., Zimmermann, C., et al. (2013). An essential role for insulin and IGF1 receptors in regulating Sertoli cell proliferation, testis size and FSH action in mice. *Molecular Endocrinology*, *27*, 814–827.
- de Rooij, D. G. (2001). Proliferation and differentiation of spermatogonial stem cells. *Reproduction*, *121*, 347–354.
- de Rooij, D. G., & Grootegoed, J. A. (1998). Spermatogonial stem cells. *Current Opinion in Cell Biology*, *10*, 694–701.
- de Rooij, D. G., & Russell, L. D. (2000). All you wanted to know about spermatogonia but were afraid to ask. *Journal of Andrology*, *21*, 776–798.
- Roosen-Runge, E. C., & Holstein, A. F. (1978). The human rete testis. *Cell and Tissue Research*, *189*, 409–433.
- Russell, L. D. (1977). Movement of spermatocytes from the basal to the adluminal compartment of the rat testis. *The American Journal of Anatomy*, *148*, 313–328.
- Sassone-Corsi, P. (2002). Unique chromatin remodelling and transcriptional regulation in spermatogenesis. *Science*, *296*, 2176–2178.
- Simoni, M., Gromoll, J., Hoppner, W., Kamischke, A., Krafft, T., Stahle, D., & Nieschlag, E. (1999). Mutational analysis of the FSH receptor in normal nad infertile men: Identification and characterization of two discrete FSH receptor isoforms. *The Journal of Clinical Endocrinology and Metabolism*, *84*, 751–755.
- Stanton, P. G. (2016). Regulation of the blood–testis barrier. *Seminars in Cell and Developmental Biology*, *59*, 166–173.

Further Reading

- Barakat, B., O'Connor, A. E., Gold, E., de Kretser, D. M., & Loveland, K. L. (2008). Inhibin, activin, follistatin and FSH serum levels and testicular production are highly modulated during the first spermatogenic wave in mice. *Reproduction*, *136*, 345–359.
- Cortes, D., Muller, J., & Skaakebaek, N. E. (1987). Proliferation of Sertoli cells during development of the human testis assessed by stereological methods. *International Journal of Andrology*, *10*, 589–596.
- Hogarth, C. A., Mitchell, D., Evanoff, R., Small, C., & Griswold, M. (2011). Identification and expression of potential regulators of the mitotic-to-meiotic transition. *Biology of Reproduction*, *84*, 34–42.
- de Kretser, D. M., Kerr, J. B., & Paulsen, C. A. (1981). Evaluation of the ultrastructural changes in the human Sertoli cell in testicular disorders and their relationship to the changes in the levels of serum FSH. *International Journal of Andrology*, *4*, 124–144.
- Nicholls, P. K., et al. (2012). Activin signalling regulates Sertoli cell differentiation and function. *Endocrinology*, *153*, 6065–6077.

"Systems Biology of Reproduction"

Spring 2024 (Even Years) – Course Syllabus
 Biol 475/575 Undergraduate/Graduate (3 Credit)
 SLN: (475) – 06763, (575) – 06764
 Time - Tuesday and Thursday 10:35 am-11:50 am
 Course Lectures in person and recorded on Canvas/Panopto and Discussion Sessions live in person and on WSU Zoom for all campuses (Hybrid Course)
 Room – CUE 418
 Course Director – Michael Skinner, Abelson Hall 507, 335-1524, skinner@wsu.edu
 Co-Instructor – Eric Nilsson, Abelson Hall 507, 225-1835, nilsson@wsu.edu
Learning Objective -
 Current literature based course on the Systems Biology of Reproduction. Learning Systems approaches to the biology of reproduction from a molecular to physiological level of understanding.

Schedule/Lecture Outline –

January	9 & 11	Week 1	Systems Biology Introduction
	16 & 18	Week 2	Molecular/ Cellular/ Reproduction Systems
	23 & 25	Week 3	Sex Determination Systems
Jan /Feb	30 & 1	Week 4	Male Reproductive Tract Development & Function
February	6 & 8	Week 5	Female Reproductive Tract Development & Function
	13 & 15	Week 6	Gonadal Developmental Systems Biology
	20 & 22	Week 7	Testis Systems Biology
	27 & 29	Week 8	Ovary Systems Biology
March	5 & 7	Week 9	Epigenetics and Transgenerational Gonadal Disease
	11 – 15	Week 10	Spring Break
	19 & 21	Week 11	Gametogenesis/ Stem Cells/ Cloning
	26 & 28	Week 12	Hypothalamus- Pituitary Development & Function
April	2 & 4	Week 13	Reproductive Endocrinology Systems
	9 & 11	Week 14	Fertilization & Implantation Systems
	16 & 18	Week 15	Fetal Development & Birth Systems
	23 & 25	Week 16	Assisted Reproduction/Contraception
Apr/May	30 & 2	Week 17	Exam or Grant Review

Spring 2024 – Systems Biology of Reproduction
 Lecture Outline – Testis Systems Biology
 Michael K. Skinner – Biol 475/575
 CUE 418, 10:35-11:50 am, Tuesday & Thursday
 February 20, 2024
 Week 7

Testis Systems Biology

- Cell Biology of the Testis
 - Cell Types and Organization
 - Cell Associations
- Spermatogenesis
 - Stages and Cycle
 - Germ Cell Differentiation
 - Genes Involved
- Endocrinology of the Testis
 - Gonadotropins
 - Testosterone and Leydig Cell
- Cell-Cell Interactions
 - Types of Interactions
 - Sertoli-Germ Cell Interactions
 - Other Cellular Interactions

Required Reading

de Kretser, et al. (2018) Structure/Cells Overview. In: Encyclopedia of Reproduction (Second Edition). Volume 1, Pages 10-16

Spring 2024 – Systems Biology of Reproduction
 Discussion Outline – Testis Systems Biology
 Michael K. Skinner – Biol 475/575
 CUE 418, 10:35-11:50 am, Tuesday & Thursday
 February 22, 2024
 Week 7

Testis Systems Biology

Primary Papers:

1. Guo, et al. (2021) Cell Stem Cell 28,764-778
2. Endo, et al. (2015) PNAS E2347-2356
3. Guo, et al. (2018) Cell Research 28:1141-1157

Discussion

Student 5: Reference 1 above

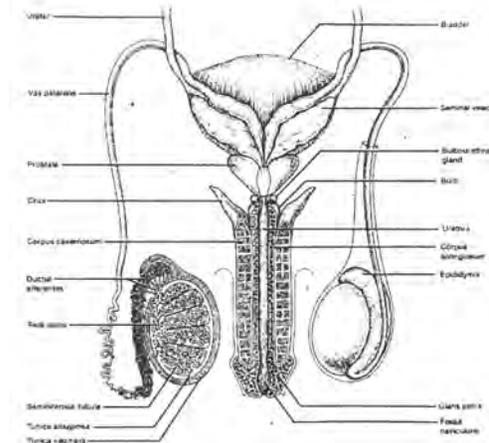
- What was the technology used?
- What experimental design was used?
- What insights were obtained on testis somatic cell and germ cell origins?

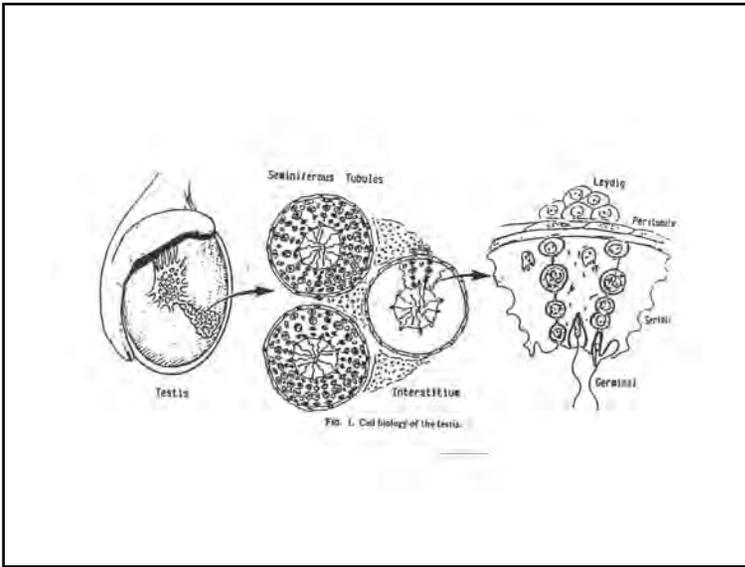
Student 6: Reference 2 above

- What was the experimental design and culture system used?
- What spermatogenic process occurred in vitro?
- How could this technology be applied?

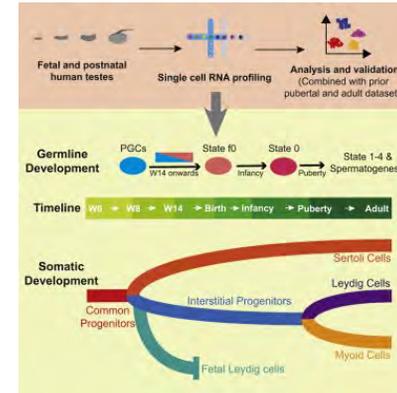
Student 7: Reference 3 above

- What is the experimental and systems approach?
- What single cell expression and epigenetic relationships exist?
- What insights are provided on testis cell biology?

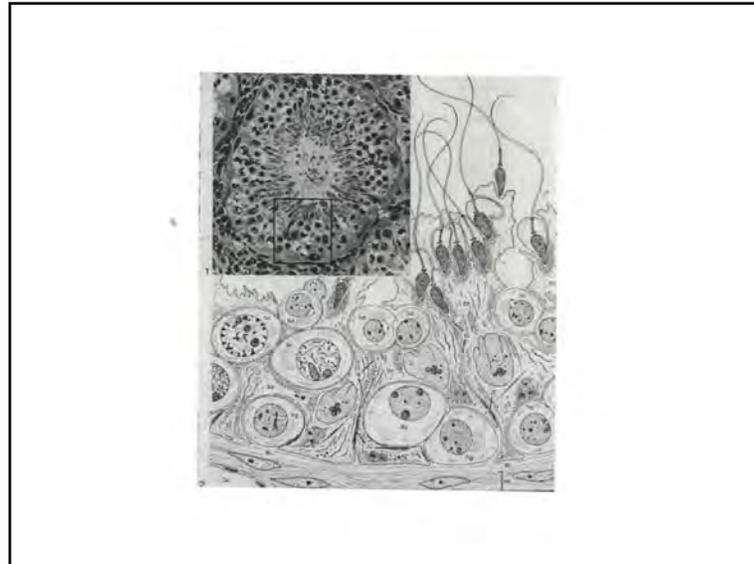
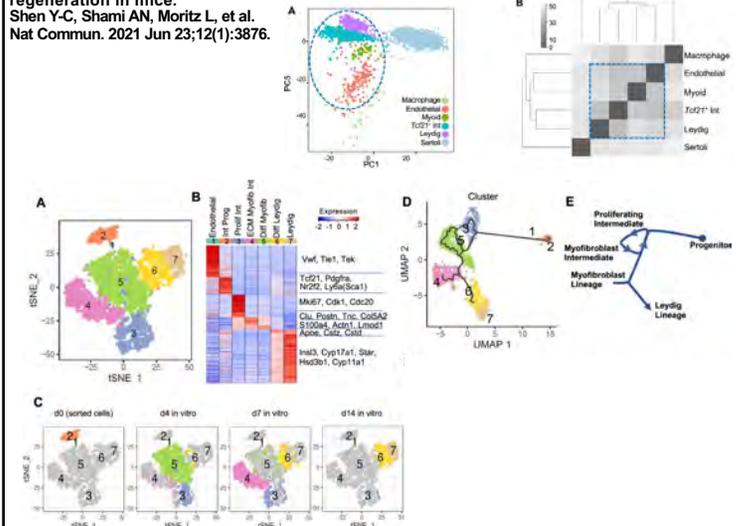




Single-cell analysis of the developing human testis reveals somatic niche cell specification and fetal germline stem cell establishment.
 Guo J, Sosa E, Chitiashvili T, et al.
 Cell Stem Cell. 2021 Apr 1;28(4):764-778.e4.

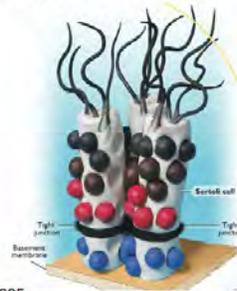


TCF21 + mesenchymal cells contribute to testis somatic cell development, homeostasis, and regeneration in mice.
 Shen Y-C, Shami AN, Moritz L, et al.
 Nat Commun. 2021 Jun 23;12(1):3876.



Sertoli cell – spans the entire seminiferous epithelium

Blood testis barrier – most exclusive component is specialized tight junctions of Sertoli cells.



Senger, 2005



Russell and Griswold, 1993

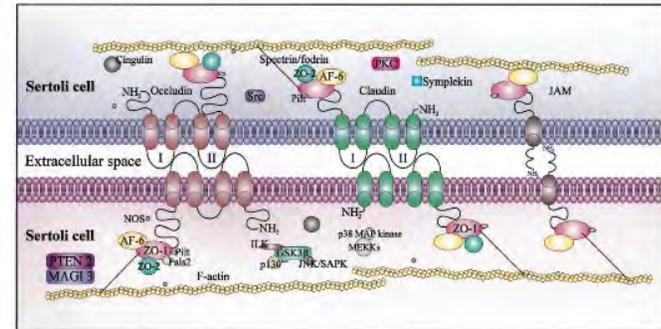
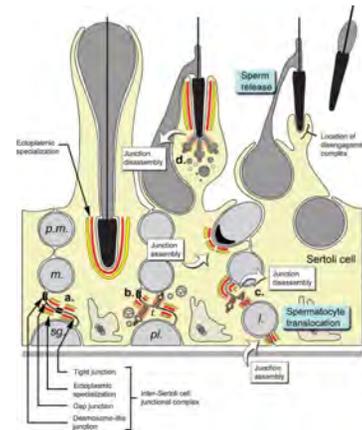
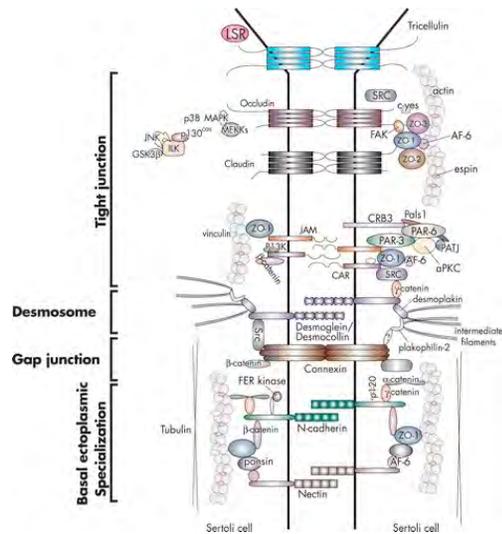


FIG. 6. Schematic drawing illustrating the molecular architecture of the three multiprotein complexes found at the Sertoli cell tight junction that constitute the blood-testis barrier. Shown are the three multiprotein complexes found at the Sertoli cell tight junction: 1) occludin -ZO-1/ZO-2; 2) claudin -ZO-1/ZO-2; and 3) JAM -ZO-1. Also shown are peripheral membrane proteins known to regulate Sertoli cell tight junction dynamics. This figure was prepared based on the following original research articles and reviews (Refs. 4, 28, 114, 149, 150, 254 -256, and 341).



Blood testis barrier creates two compartments

Basal and adluminal side

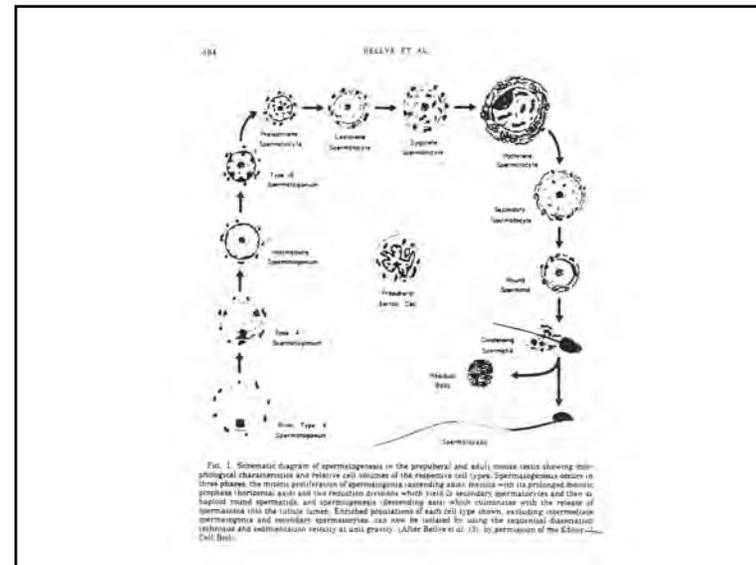
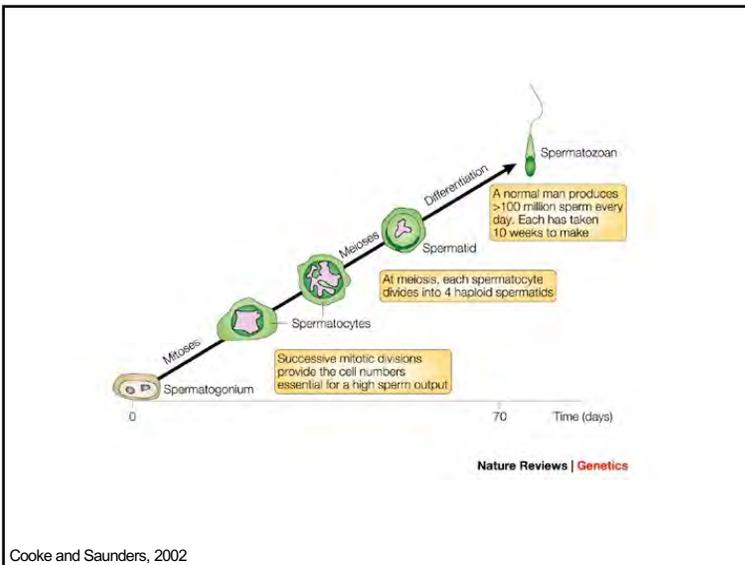
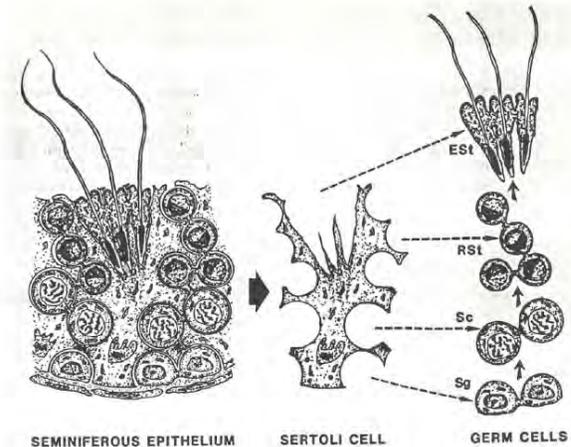
Basal – Spermatogonia, primary spermatocytes

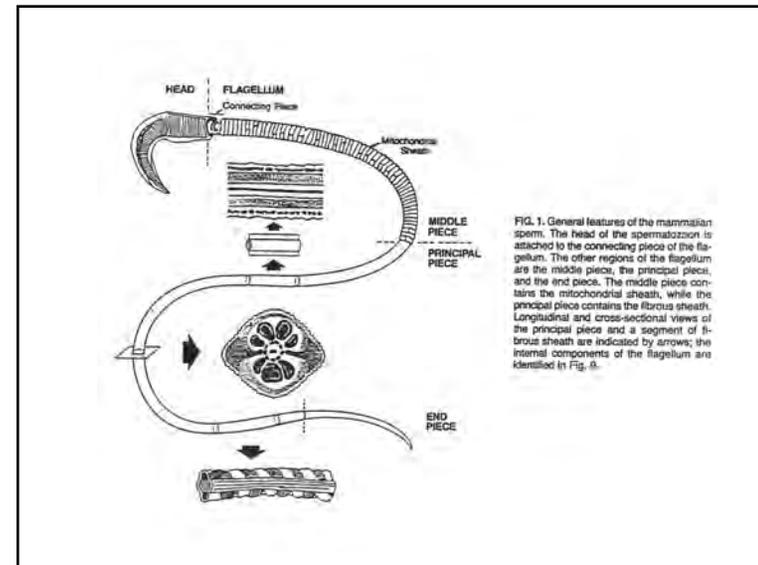
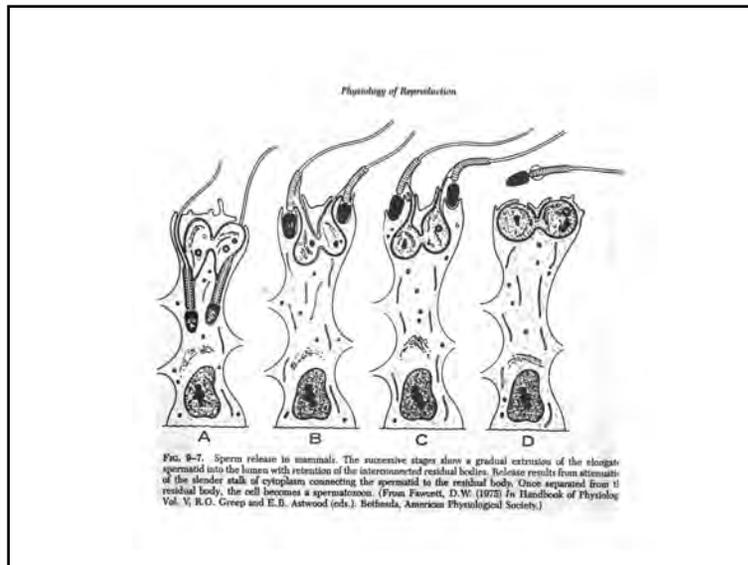
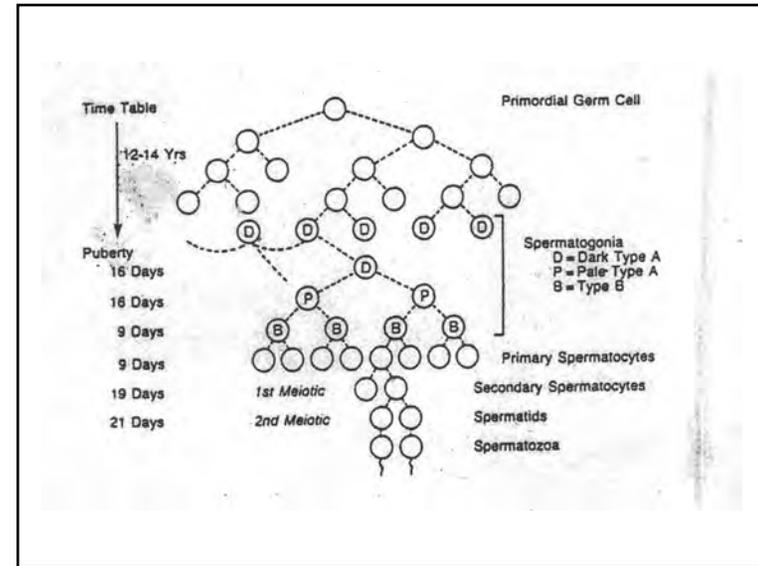
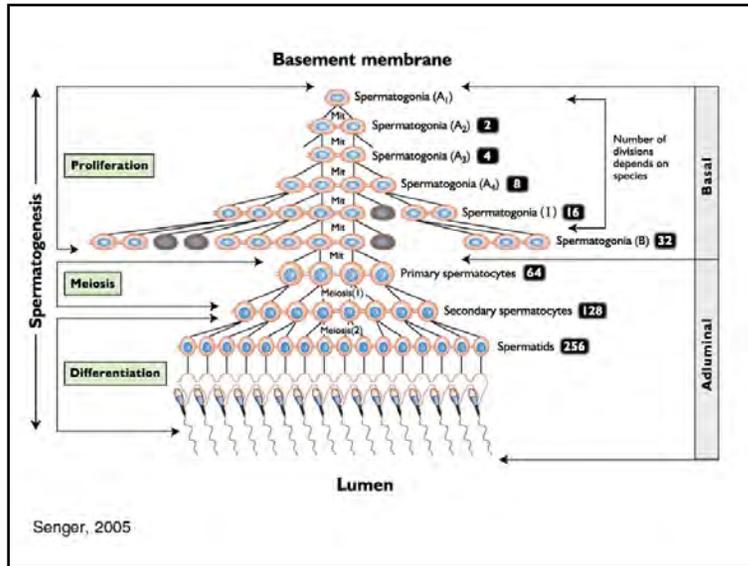
Primary spermatocytes

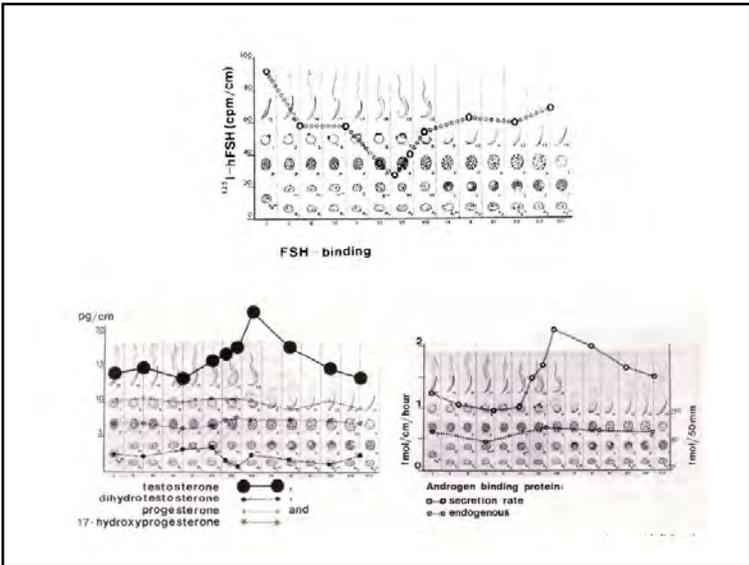
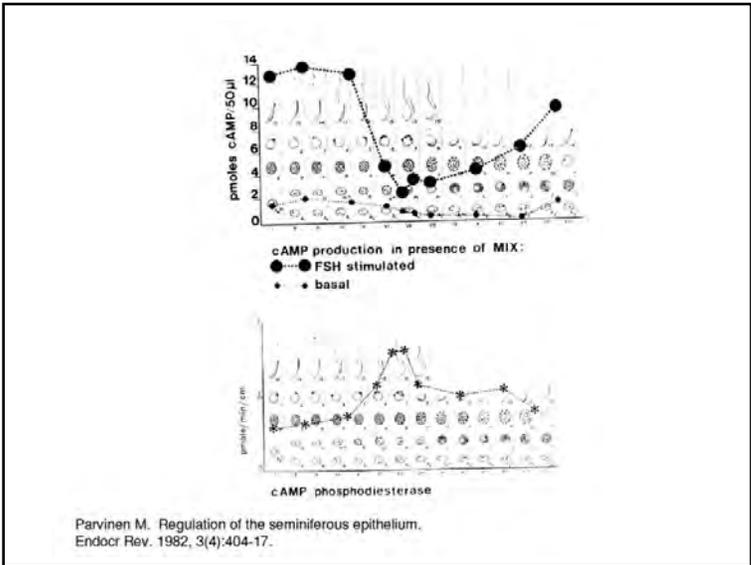
migrate through tight junctions so secondary spermatocytes and spermatids are located in adluminal side

Seminiferous epithelium – Sertoli and differentiating germ cells of the seminiferous tubule

Cells of the seminiferous epithelium





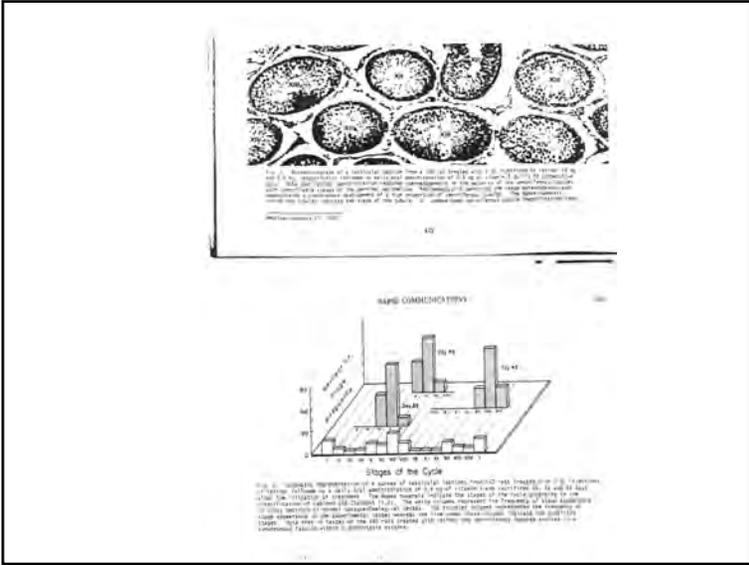


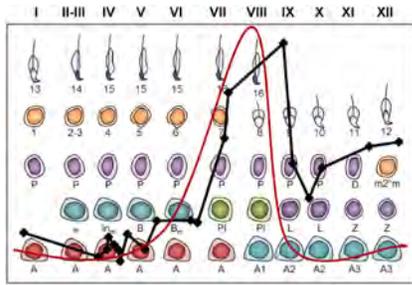
Vitamin A deficient (VAD) model
 Vitamin A stored in the liver.

Rat – put on VAD diet at 20 days of age. In 9-10 weeks spermatogenesis has stopped. Only germ cells present are type A spermatogonia and a few preleptotene spermatocytes

Inject rat with retinol and return to a normal diet and spermatogenesis resumes.
 Spermatogenesis is synchronous for 2-3 rounds.

Sacrifice animals at specific times can get testis samples at 1-2 stages. Gene and protein expression associated with select stages.





Max. 35 pmol/g testis
 Min. 3 pmol/g testis
 WT 8-12 pmol/g testis
 Half-life of RA is 1.3 ± 0.1 h in the testis

Retinoic acid pulse across the cycle of the seminiferous epithelium. Red line shows the predicted RA pulse based on previous data, black line shows actual, relative RA values across the cycle in stage-synchronized testes (data from Z1 RA levels peak at late stage VIII/early stage IX and reach a maximum value of 35 picomoles per gram testis (pmoles/g testis). Minimum RA values around 3 pmoles/g testis were observed in stages II through V. In unsynchronized adult testes where all stages of the seminiferous epithelium are present, RA values averaged around 8-12 pmoles/g testis

Retinoic acid metabolism links the periodical differentiation of germ cells with the cycle of Sertoli cells in mouse seminiferous epithelium.

Sugimoto R, Nabeshima Y, Yoshida S. Mech Dev. 2012 Jan-Feb;128(11-12):610-24.

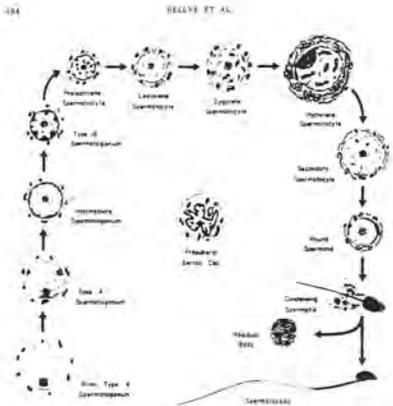
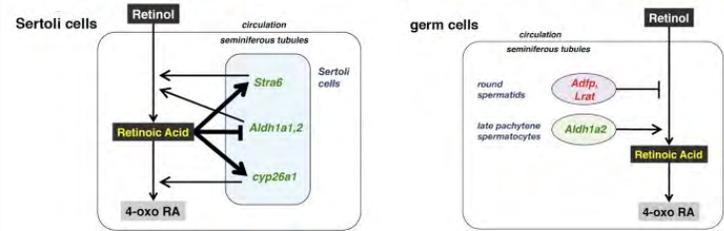


Fig. 1. Schematic diagram of spermatogenesis in the prepubertal and adult mouse testis showing morphological characteristics and relative cell numbers of the meiotic cell cycle. Spermatogenesis occurs in three phases: the mitotic proliferation of spermatogonia (ascending axis) results with its prolonged mitotic complete horizontal axis and the meiotic division which yields 2 secondary spermatocytes and then 4 haploid round spermatids, and spermiogenesis (descending axis) which culminates with the release of spermatozoa into the lumen. Enriched populations of each cell type shown, excluding spermatogonia, spermatocytes and secondary spermatocytes, can now be obtained by using the regional dissociation technique and subsequent velocity of unit gravity. (After Bellve et al. (3), by permission of the Society of Cell Biol.)

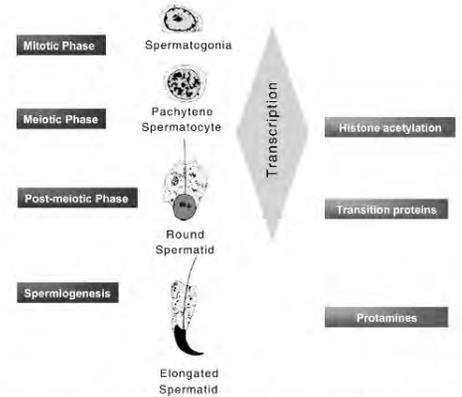


Figure 1. Spermatogenesis follows a carefully orchestrated differentiation program that depends on transcriptional regulation. The transcription phase begins in spermatogonia. Meiotic cells have a high level of transcription that is promoted by histone acetylation, making DNA accessible. Elevated transcription in meiotic cells is required for chromosome congression, recombination and desynapsis. In post-meiotic spermatids there is a second round of histone acetylation and transcription, followed by the sequential replacement of histones by transition proteins then protamines. This altered chromatin architecture leads to arrested transcription and differentiation into elongated spermatozoa.

Reproductive (2006) 128:5-12. www.reproductivebiology.org

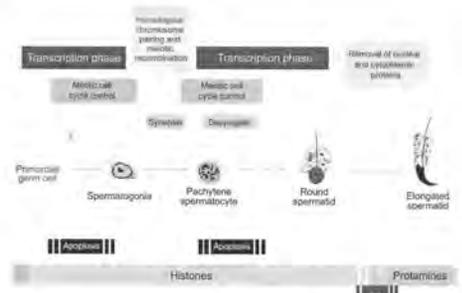


Fig. 1. Spermatogenesis is a cyclic developmental process by which spermatogonia cells generate the mature spermatozoon. These events are characterized by important modifications in chromatin organization, basically during two periods, meiosis—which includes the synapsis and desynapsis of the chromosomes—and the histone-protamine transition. Postmeiotically, a powerful wave of transcription occurs in haploid cells, which is governed by highly specialized molecular mechanisms. Specific genes operate at distinct steps of the spermatogenic process.

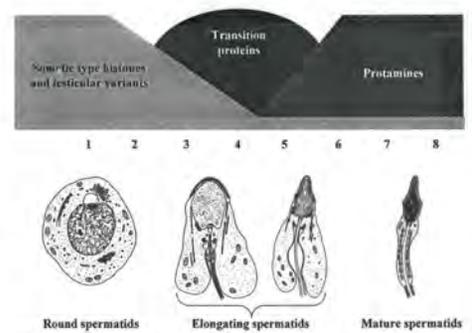


FIG. 2 Schematic diagram of histone-to-protamine replacement during human spermiogenesis that is subdivided into eight successive steps. Both somatic-type and testis-specific histones present in round spermatids are, for the most part, displaced by transition proteins at the beginning of the elongation phase. However, a low amount of somatic-type histones persists in late spermatids and mature spermatozoa.

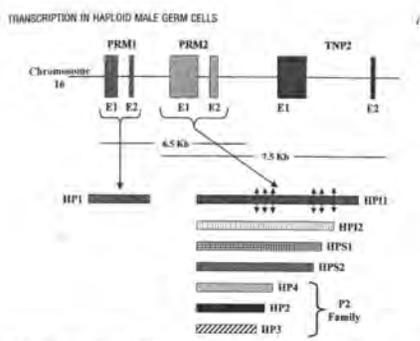


FIG. 3 The genes coding for PRM1, PRM2, and TNP2 are closely linked over a stretch of DNA 13–15 kb long. Whereas *Prm1* encodes directly for the 50-amino-acid-long HP1 protein, *Prm2* codes for a 101-amino-acid-long precursor, HP1. The latter, actually referred to as human proprotamine pH2, is posttranslationally processed in several steps (HP2, HP3, HP4, HP5, successively) via proteolytic cleavage at specific sites leading from HP1 to protamine HP3, E, exon.

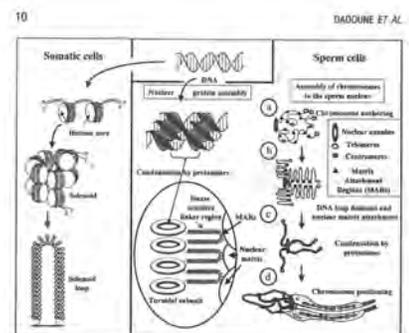


FIG. 5 Comparative chromatin organization in somatic and sperm cells. In somatic cells, DNA is bound in octamers of histone proteins forming the nucleosomal fiber. Chromatin is then coiled into a 30-nm solenoid filament which is further organized into loop domains. The latter play an important role in DNA replication and gene transcription. In sperm cells, chromosomal DNA is organized into loop domains attached at their bases to the nuclear skeleton, a round-shaped structure located at the nuclear periphery (a) and to the nuclear matrix, by specific sequences termed matrix attachment regions (MARs) (b). The postmeiotic-bound DNA is coiled into thread-like structures whose MARs are comprised of DNase-sensitive linker regions disposed between them (c). Chromosome positioning is the final step in chromatin organization (i) (Drawn from data provided by Sotolongo et al., 2003; Ward, 1993, 1997).

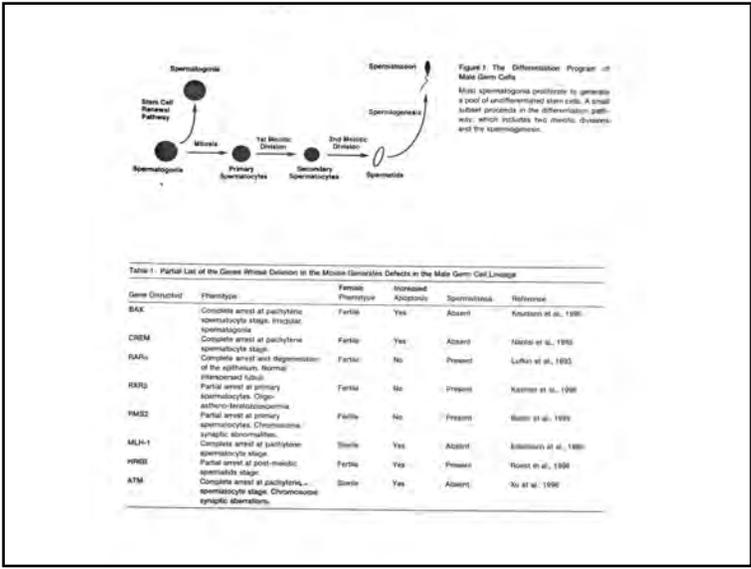
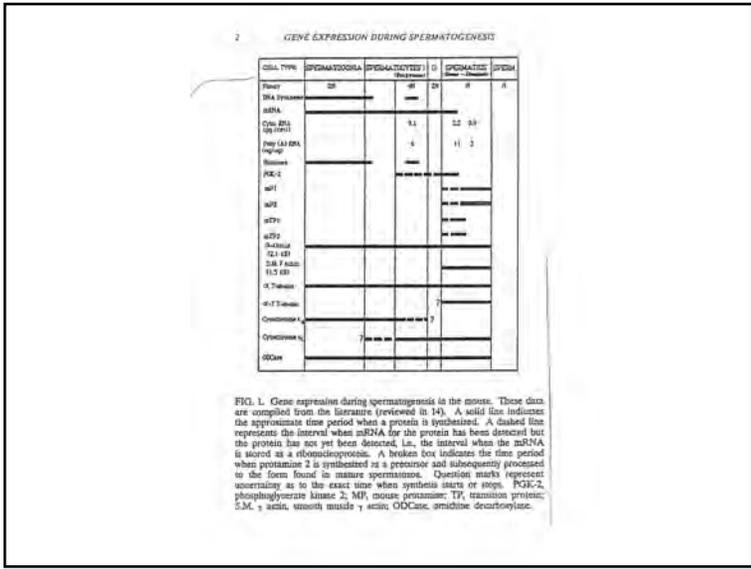
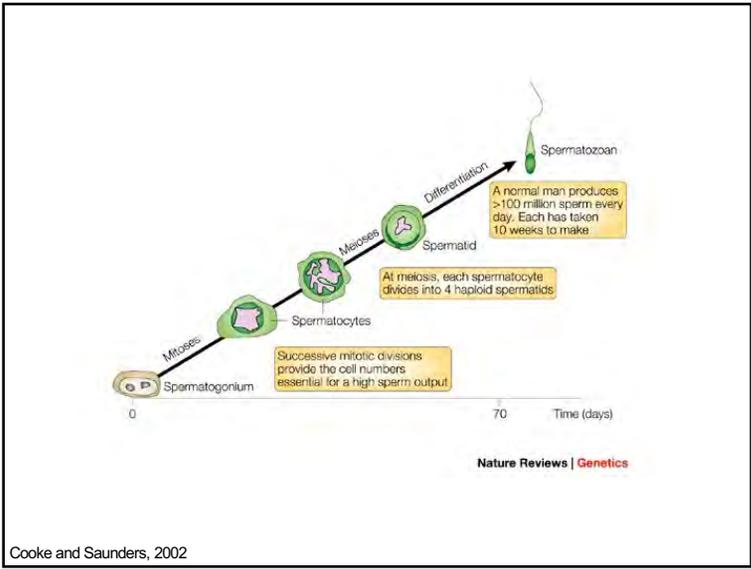


Table 3 | Genes implicated in germ-cell meiosis by mouse knockout studies

Gene	Function	KO phenotype	End meiotic stage	References
<i>Atm</i>	PI3 kinase	Male and female sterility	Leptonema/pachytene	31
<i>Atr</i>	PI3 kinase	Embryonic lethal	N/A	33
<i>Rac2f1</i>	RacA-like	Embryonic lethal	N/A	118
<i>Dmc1</i>	RecA-like	Male and female sterility	Zygotene	26,27
<i>Msh4</i>	Mismatch repair	Male and female sterility	Zygotene	117
<i>Msh5</i>	Mismatch repair	Male and female sterility	Zygotene	118
<i>Mlh1</i>	Mismatch repair	Male and female sterility	Post-pachytene	119
<i>Prps2</i>	Mismatch repair	Male and female sterility	Leptonema/pachytene	120
<i>H2afx</i>	DSB recognition	Male sterile/genomic instability	Pachytene	36
<i>Spo11</i>	DSB formation	Male/female sterility	Leptonema/zygotene	24,25
<i>Scp3</i>	Axial element formation	Male sterile/female fertile	Zygotene	114
<i>Mlh3</i>	Mismatch repair	Male/female sterility	Metaphase I arrest: aneuploidy	115

Adapted from REF 21. *Atm*: ataxia telangiectasia mutated homologue; *Atr*: ataxia telangiectasia and rad3-related; *Dmc1*: despaired meiotic cDNA 1 homologue; *DSB*: double-stranded break; *H2afx*: H2A histone family, member X; *Mlh1/3*: mismatch homologue 1/3; *Msh4/5*: mismatch homologue 4/5; *Prps2*: post-meiotic spermatogenesis associated 2; *Rac2f1*, *RAD51* homologue; *Scp3*: synaptonemal complex protein 3; *Spo11*: sporulation protein, meiosis-specific; *SP011* homologue; *N/A*, not applicable; *KO*, knockout.

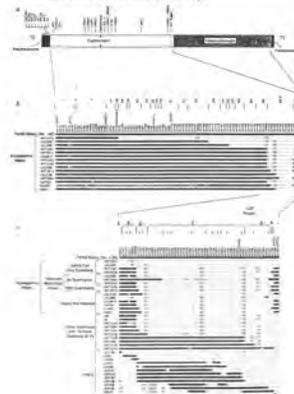
TABLE 2. Representative RNA-binding Proteins of the Testis*

TB-RBP (36, 37, 39, 62)
 p48/52 (55, 61)
 53 and 55 kDa (40)
 Prbp (41)
 Spnr (42)
 Tenr (43)
 RBM (44, 72)
 DAZ (44, 73)
 SOD-RBP (50)
 DBY (71)
 EIFIAY (71)
 ATP-dependent RNA-helicases (74, 75)
 Poly (A) Binding Protein (76, 77)

*This table lists some of the currently identified RNA-binding proteins expressed, but often not solely expressed in the testis. It does not include many ubiquitous RNA-binding proteins that also function during spermatogenesis.

Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene

Rivar Rigoir, Yun-Yi Lee, Fu-Sai Li, Rajni Akhgar, Laura G. Brown, Michael Rosenfeld, Steve Rozen, Tom Jaffe, Donald Strawn, Orit Benayahu, Albert de la Cueva, Sherman Silber & David C. Page



ONCOGENES AND SPERMATOGENESIS

Oncogene	Function	Localization
c-fos	nuclear proto-oncogene	spermatogonia
c-jun	nuclear proto-oncogene	spermatogonia, early spermatocyte
c-myc	nuclear proto-oncogene	spermatogonia, early spermatocyte
c-raf	serine/threonine kinase	spermatogonia, spermatocytes, round spermatids
c-ras	membrane GTP binding proteins	all stages
c-ras ^H		spermatocytes
c-ras ^K		all stages
c-mos	serine/threonine kinase	round spermatids
c-abl	membrane tyrosine kinase	early spermatids
pim-1	protein kinase	early spermatids



Spermatogonia - Spermatocyte - early spermatide - late spermatide
 meiosis

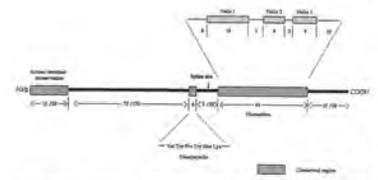


FIGURE 1. Chromatid bands of centromere the p-arms. Chromatid bands are indicated by boxed lines; centromere between p-arms and centromere of the q-arms. The short arm (Yp) is marked by the centromere and the long arm (Yq) is marked by the centromere. Yp11, Yp12, and Yp13 are the centromere bands.

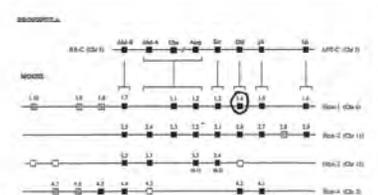
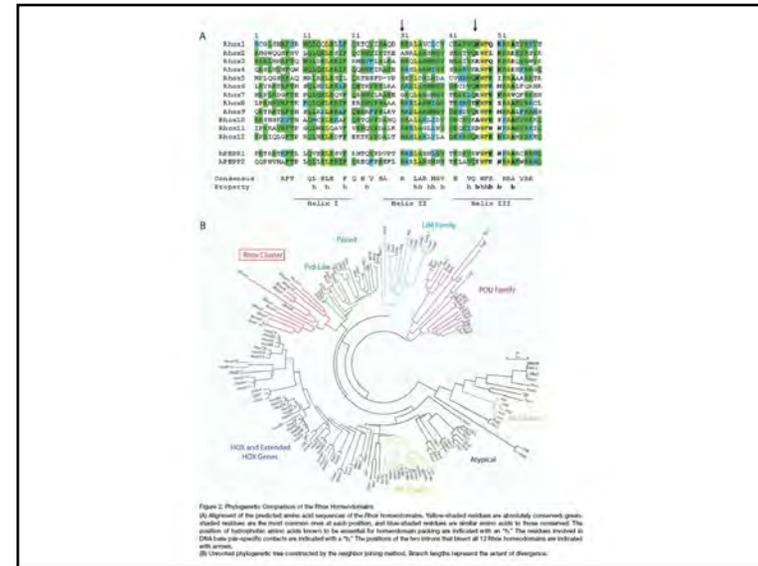
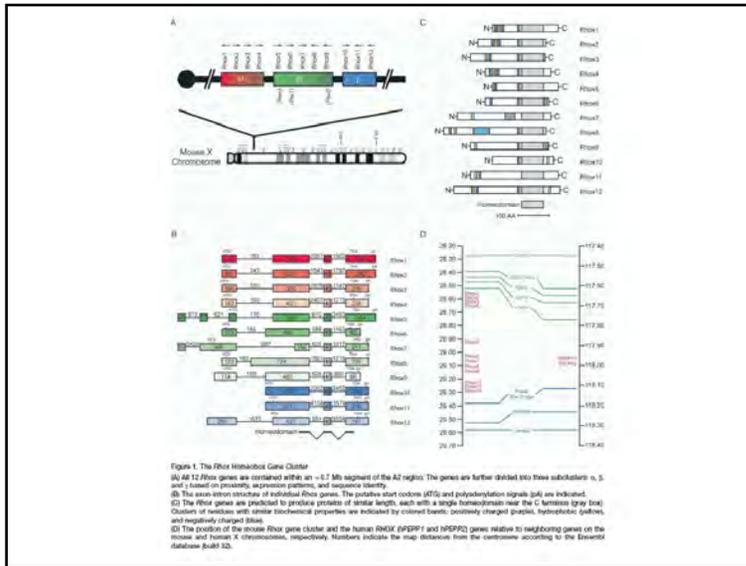


FIGURE 2. Stages of the spermatogenesis and localization of various oncogenes. The localization of various oncogenes is indicated by the boxes in the corresponding column. The boxes in the corresponding column indicate the localization of various oncogenes. The boxes in the corresponding column indicate the localization of various oncogenes.



Small RNAs just got bigger: Piwi-interacting RNAs (piRNAs) in mammalian testes

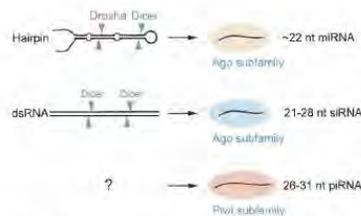


Figure 1. Classification of small RNAs. Definition and classification of small RNAs conventionally relies on their biogenesis mechanism. Two relatively well-defined classes of small RNAs include microRNAs (miRNAs) and small interfering RNAs (siRNAs). The biogenesis mechanism for piRNAs is currently unknown.

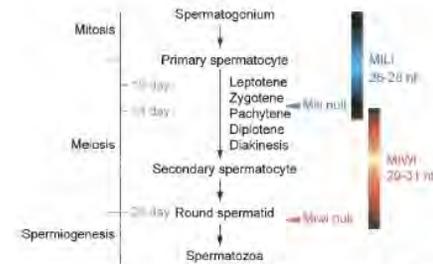


Figure 2. Expression of piRNAs and Piwi subfamily members during mouse spermatogenesis. Mammalian spermatogenesis occurs within the seminiferous epithelium inside the seminiferous tubule and can be divided into three phases.

Hypogonadal – pituitary – testis axis

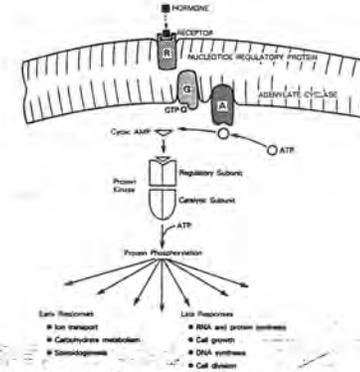
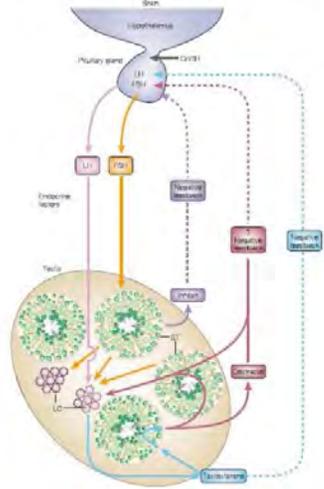


Figure 4-15 Hormonal stimulation of adenylate cyclase in peptide-regulated target cells. Increased production of cyclic adenosine monophosphate (cAMP) leads to activation of protein kinase and phosphorylation of regulatory protein subunits involved in the early and late aspects of hormone action.

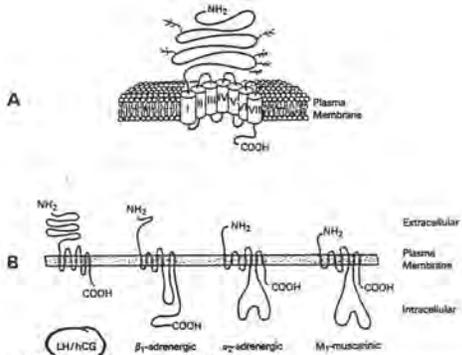


Figure 4-16 Structure of the luteinizing hormone (LH) receptor.
 A. General structure of extracellular region and transmembrane domain.
 B. Comparison of LH receptor with other G-protein-coupled receptors indicating the differences in extracellular regions and third intracellular loops.

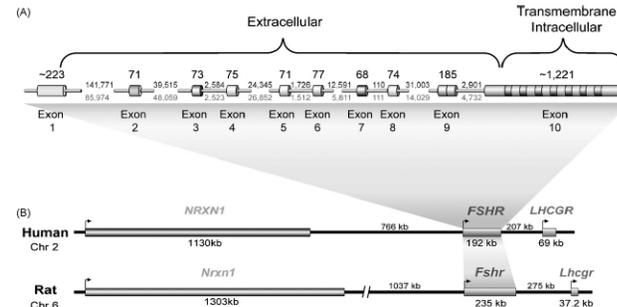
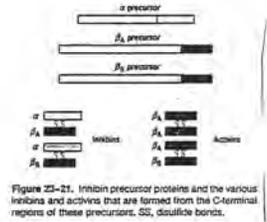
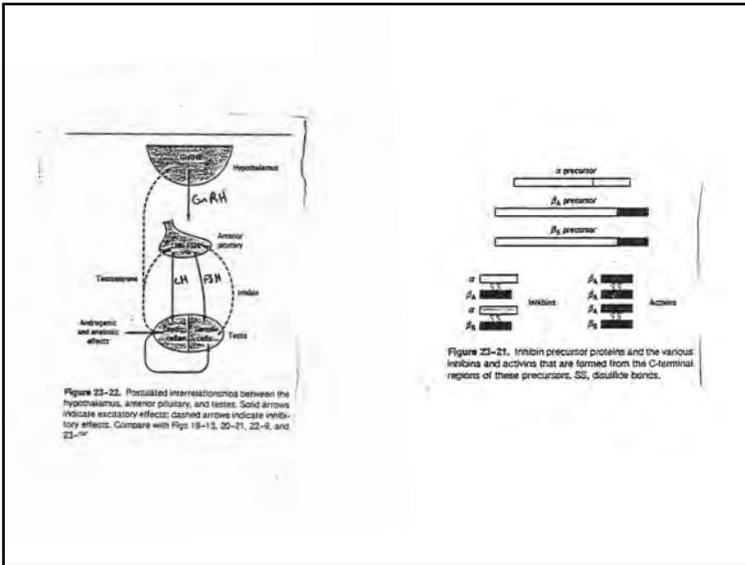


Fig. 1. *Fshr* gene structure and chromosomal location. (A) The exon-intron structure of *Fshr* corresponds to the domain structure of the protein. Exons 1 through 9 code for the extracellular ligand binding domain while exon 10 codes for the transmembrane and intracellular domains. Each of the small exons 2 through 8 encode individual leucine rich repeats and exon 9 codes for 2. The size of each exon in base pairs is shown above the diagram and the intron sizes are noted for rat (above) and human (below) between each exon. (B) In addition to the gene structure, synteny in the chromosomal environment surrounding *Fshr* is conserved between species. The size of *Fshr* and neighboring genes in humans and rats is shown above each gene and intergenic distances are noted. Adapted with permission from Heckler (2005) (Copyright 2005, Elsevier Academic Press).



Testosterone (DHT)

50-100 ng/ml in testis

2-5 ng/ml in serum

Androgen receptors are saturated ~25 ng/ml

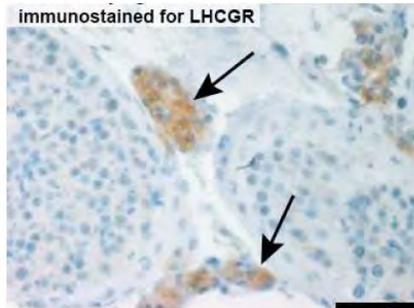
Produced by Leydig cells (LH regulation)

AR expressed: Sertoli, peritubular myoid, Leydig, endothelial cells of blood vessels, germ cells?

Interstitial cells – Leydig cells

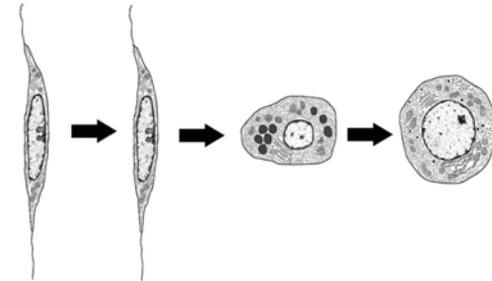
Receptors for LH
Produce testosterone

Kossack et al. PLoS Med 5(4)



De Novo Testosterone Production in Luteinizing Hormone Receptor Knockout Mice after Transplantation of Leydig Stem Cells.
Lo et al, 2004, Endo 145:4011

Leydig cell differentiation: stem to adult cells.



Stem Leydig Cell	Progenitor Leydig Cell	Immature Leydig Cell	Mature (Adult) Leydig Cell
Age: 7 d	Age: 21 d	Age: 35 d	Age: 90 d
Morphology: Spindle	Morphology: Spindle	Morphology: Round with Lipid Droplets	Morphology: Round without Lipid Droplets
T: No	T: Low	T: Higher	T: Highest
T Metabolites: No	T Metabolites: High	T Metabolites: Highest	T Metabolites: Low
LH Receptor: No	LH Receptor: Low	LH Receptor: Intermediate	LH Receptor: High
Mitotic Activity: Low	Mitotic Activity: High	Mitotic Activity: Low	Mitotic Activity: Probably none

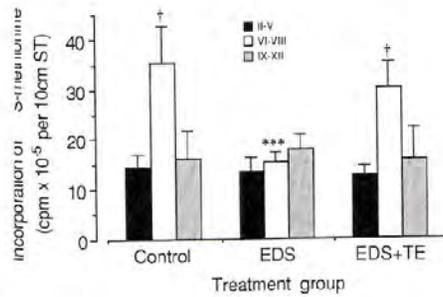
Investigation of testosterone regulation of spermatogenesis

Treat rats with ethane dimethane sulfonate (EDS)
selectively kills I evdia cells in rats

Protein production:
Stages II-V: no
change

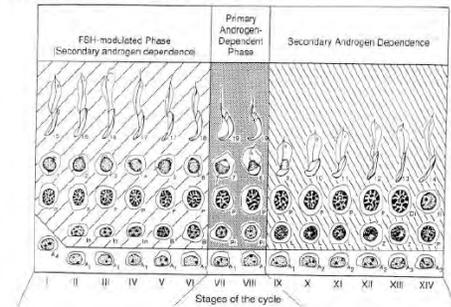
Stages VI-VIII:
significantly
suppressed

Stages IX-XII: no
change



Degeneration of germ cells at stage VII (~4 days)
progressive increase

Day 8 post injection – 80% step 10-12 spermatids
degenerating



Testosterone supports germ cell differentiation during stages VII-VIII

Loss of germ cells – progressive loss
EDS – rescued by testosterone treatment

Identification, proliferation, and differentiation of adult Leydig stem cells.

Stanley E, Lin CY, Jin S, Liu J, Sottas CM, Ge R, Zirkin BR, Chen H.
Endocrinology. 2012 Oct;153(10):5002-10.

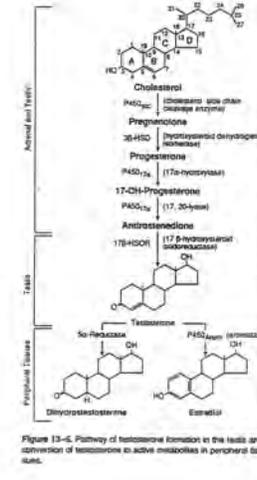
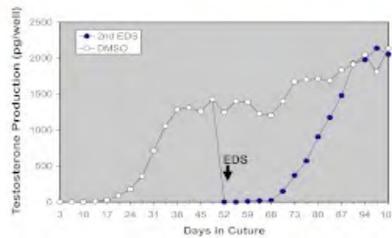


Figure 13-6. Pathway of testosterone formation in the testis and conversion of testosterone to active metabolites in peripheral tissues.

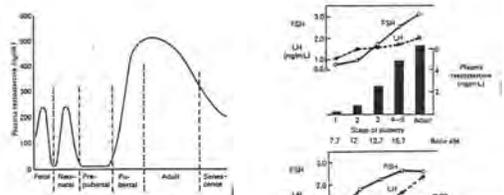


Figure 23-18. Plasma testosterone at various ages in human males.

Table 23-4. Body changes at puberty in boys (male secondary sex characteristics).

External genitalia: Penis increases in length and width. Scrotum becomes pigmented and rugose.

Internal genitalia: Seminal vesicles enlarge and secrete and begin to form prostatic, prostatic and bulbourethral glands enlarge and secrete.

Voice: Larynx enlarges, vocal cords increases in length and thickness, and voice becomes deeper.

Hair growth: Beard appears, hairs on axilla recedes anterolaterally. Pubic hair grows with male (straight with slight up) pattern. Hair appears in armpits, on chest, and around anus; genital body hair increases.

Muscle: More aggressive, active attitude, interest in opposite sex develops.

Body conformation: Shoulders broaden, muscles enlarge. Skin: Sebaceous gland secretion thickens and increases (contributes to acne).

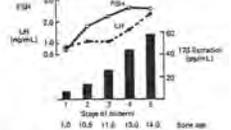
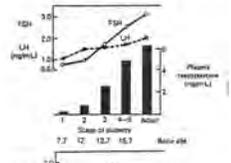
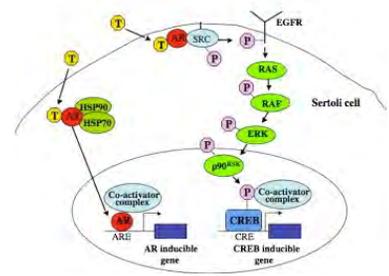


Figure 23-8. Changes in plasma hormone concentrations during puberty in boys (top) and girls (bottom). Stage 1 of puberty is prepubescence in both sexes. In boys, stage 2 is characterized by beginning enlargement of the testes, stage 3 by penis enlargement, stage 4 by growth of the sperm penis, and stage 5 by adult genitalia. In girls, stage 2 is characterized by breast buds, stage 3 by areolar and enlargement of the breasts, stage 4 by development of the areolas, and stage 5 by adult breasts (reproduced, with permission, from Guadagnoli MM: Onset of puberty. In: Puberty: Biologic and Psychosocial Correlates. Baltimore MD (Dove), and Scientific Knowledge BV, 1975).

Classical and Non-classical Testosterone Pathways



Classical and non-classical pathways of testosterone action. The classical pathway of testosterone action is shown on the left. Testosterone diffuses through the plasma membrane and interacts with AR sequestered in the cytoplasm with heat shock proteins (HSP). As a result, the AR undergoes a conformational change, is released from the HSPs and then travels to the nucleus due to an intrinsic nuclear localization domain. In the nucleus AR binds to specific DNA motifs (AREs) and recruits co-activators or co-repressors (not shown) to regulate testosterone-mediated transcription. The non-classical pathway shown in the center and on the right is initiated with testosterone binding to the classical AR either localized near the plasma membrane or in the cytoplasm. AR then interacts with and causes the phosphorylation (P) of Src kinase, which may be tethered to the plasma membrane or present in membrane-associated protein complexes. The activated Src then phosphorylates the epidermal growth factor receptor (EGFR) directly or via intermediary factors. The EGFR then activates the MAP kinase cascade likely through the Ras small G protein that causes the phosphorylation of Raf kinases that activate MEK kinase that in turn activates ERK kinase. ERK then activates the p90RSK kinase to phosphorylate CREB on serine 133 allowing CREB bound to cAMP response elements (CREs) to recruit coactivators and induce gene transcription. It should also be noted that the kinases that are activated by the non-classical pathway are capable of phosphorylating other spermatogenesis-regulating proteins in Sertoli cells as well as activating other transcription factors to regulate additional webs of gene expression.

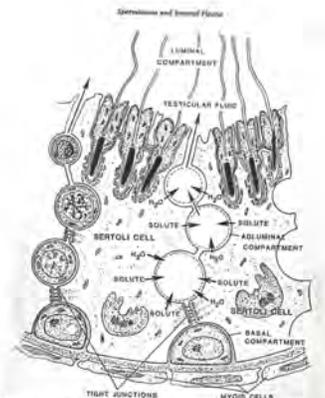


Fig. 23-11. The location of the blood-testis barrier and the compartmentalization of the testes between adjacent germinal cells. The specialized tight junctions between adjacent Sertoli cells form the principal permeability barrier between the blood and testis of most farm mammals. These so-called junctions separate the apical and basolateral membranes of Sertoli cells into the basal and adluminal compartments. The basal compartment is composed of the seminiferous tubule and the spermatozoa. The adluminal compartment contains the late and advanced stages of spermatozoa and the spermatozoa. The adluminal compartment communicates freely with the lumen of the seminiferous tubule. The secretion of Sertoli cells probably occurs across a region of tight junctions. The Sertoli cells are thought to pump active ions into the adluminal compartment thereby creating an osmotic gradient that would cause water to follow. The osmotic force of fluid would provide the force to pump fluid into the adluminal compartment to make up the osmotic losses at the blood-testis barrier. (From Fauci, 1978 in: Harrison's Principles of Internal Medicine, Vol. 9, Male Reproductive System, R.D. Gray and E.S. Aronoff (Eds.) Washington D.C.: American Physiological Society.)

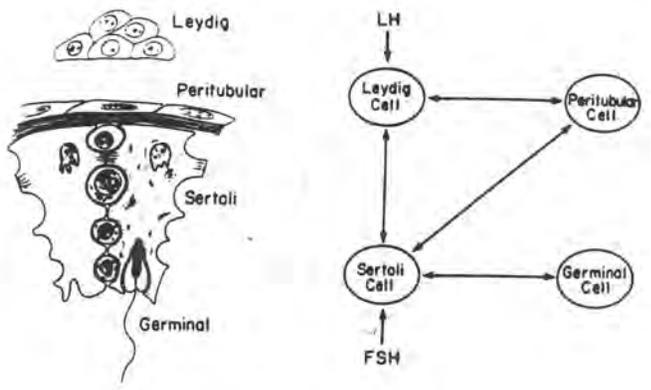


TABLE 1. Categorization of cell-cell interactions

Classification	Definition	Examples/mediators
Environmental	Interactions that influence the extracellular environment of the cell to affect cell contacts and cytoarchitecture	Extracellular matrix; cell adhesion molecules
Nutritional	Interactions involved in the delivery of essential nutrients between cells	Transfer of energy metabolites, metals, or vitamins
Regulatory	Agents provided by a cell that through a signal transduction event regulates another cell's function on a molecular level	Paracrine/autocrine factors; growth factors; differentiation factors; cytokines

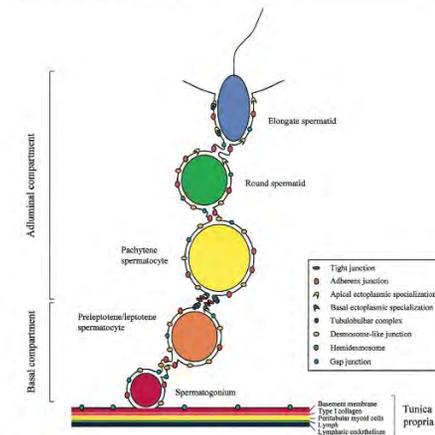
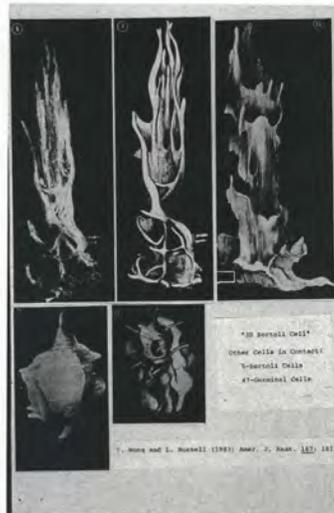
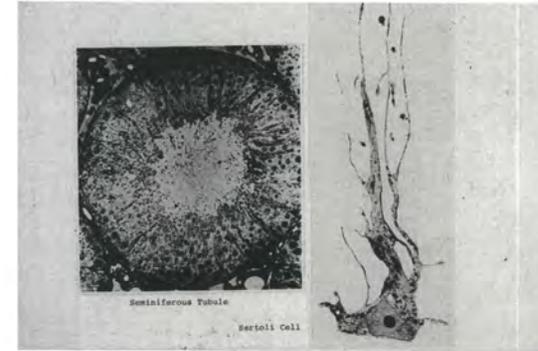


Fig. 1. Schematic drawing illustrating the relative locations of the different types of junctions found in the testis. Shown are tight, anchoring, and gap junctions. Also depicted are two kinds of specific anchoring junction types: the ectoplasmic specialization and tubulobulbar complex. Tight junctions, basal ectoplasmic specializations, and tubulobulbar complexes (not associated with blood vessels) barrier divide the seminiferous epithelium into a basal and adluminal compartment. Also shown is the proximity of the blood vessels (artery) to the basement membrane. This figure was prepared based on the following original research articles and reviews (Refs. 4, 6, 16, 18, 49, 112, 113 and 115).

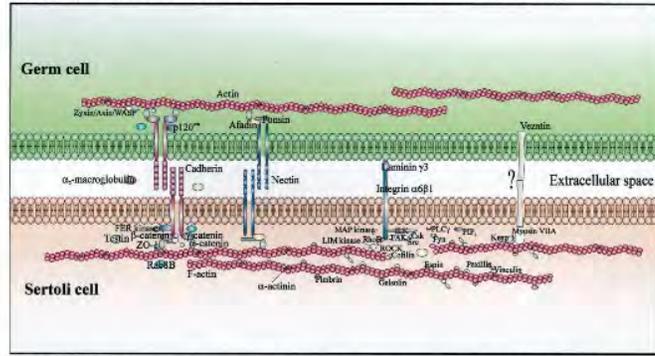
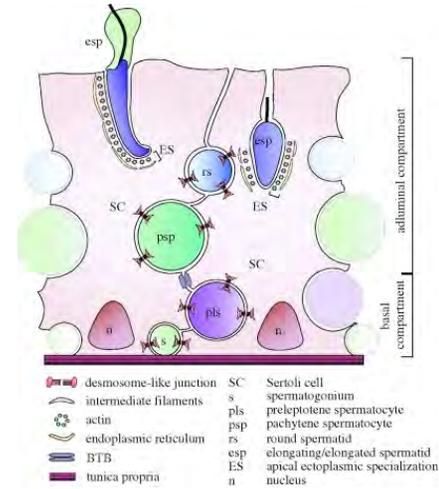


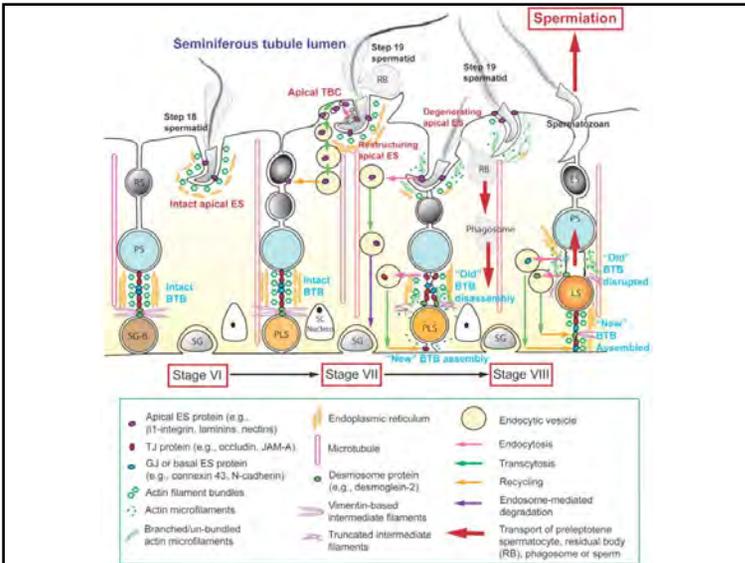
Fig. 3. Schematic drawing illustrating the molecular architecture of the four multiprotein complexes found at the Sertoli-germ cell adherens junction. Shown are the four multiprotein complexes found at the Sertoli-germ cell adherens junction: 1) cadherin-catenin, 2) afadin-afadin, 3) integrin-laminin, and 4) vezartin-myosin (7). It is not yet known whether vezartin, the binding partner of myosin, is present in the testis. Also shown are signaling proteins known to regulate Sertoli-germ cell adherens junction dynamics. This figure was prepared based on the following original research articles and reviews: Refs. 4, 48, 53, 60, and 459.

Sertoli-germ cell junctions in the testis: a review of recent data.

Kopera IA, Bilinska B, Cheng CY, Mruk DD. Philos Trans R Soc Lond B Biol Sci. 2010 May 27;365(1546):1593-605.



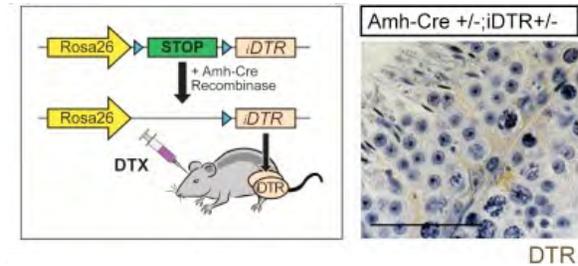
- desmosome-like junction
- intermediate filaments
- actin
- endoplasmic reticulum
- BTB
- tunica propria
- SC Sertoli cell
- s spermatogonium
- pls preleptotene spermatocyte
- psp pachytene spermatocyte
- rs round spermatid
- esp elongating/elongated spermatid
- ES apical ectoplasmic specialization
- n nucleus

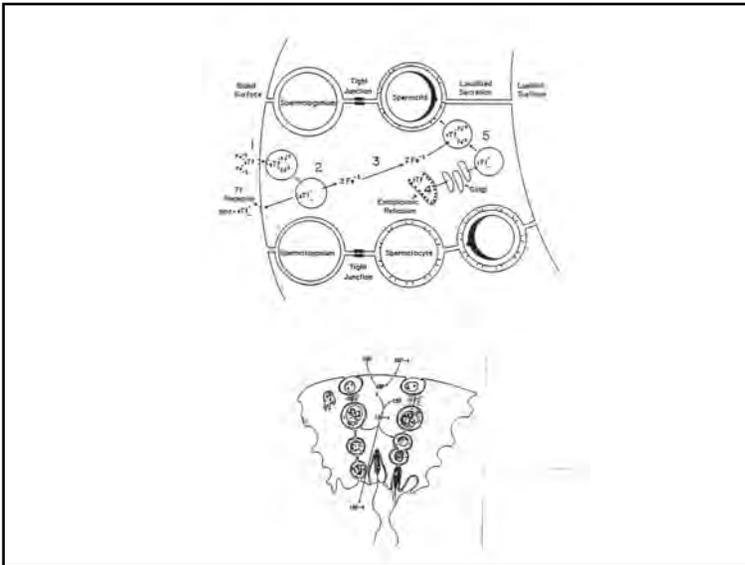


- Apical ES protein (e.g., [1]-integrin, laminins, nectin)
- TJ protein (e.g., occludin, JAM-A)
- GJ or basal ES protein (e.g., connexin 43, N-cadherin)
- Actin filament bundles
- Actin microfilaments
- Branched/bundled actin microfilaments
- Endoplasmic reticulum
- Microtubule
- Desmosome protein (e.g., desmoglein-2)
- Vimentin-based intermediate filaments
- Truncated intermediate filaments
- Endocytic vesicle
- Endocytosis
- Transcytosis
- Recycling
- Endosome-mediated degradation
- Transport of preleptotene spermatocyte residual body (RB), phagosome of sperm

Sertoli cells maintain Leydig cell number and peritubular myoid cell activity in the adult mouse testis.

Rebourcet D, O'Shaughnessy PJ, Monteiro A, et al. PLoS One. 2014 Aug 21;9(8):e105687.





February 2004 CELL-CELL INTERACTIONS IN THE TESTIS 47

Table 1. Male Sertoli cell secretory products

Secretory product	Function and/or characteristics	Potential overexpression
Transmembrane proteins		
ABP 100-427	Androgen transport/oxidation	
Transferrin (2)	Iron transport	
Conopseaquin 164	Copper transport	
Sialoadhesin	Sialoglycanic lectin	ST2-981
Test-1 (93-47)	Test-1	Test-1 (70)
		Transferrin (71)
Proteases/inhibitors		
Fibrinogen activator (72, 73)	Activator	Androgen (76)
Cysteine proteinase-2 (75-79)	Cysteine protease	(77)
α-Macroglobulin (8)	Protease inhibitor	(8)
Extracellular matrix components		
Laminin (78)	Extracellular matrix component	
Collagen IV acid (79)	Extracellular matrix component	
Perlecanin (80)	Extracellular matrix component	
Growth factors		
TGF-β (81)	Growth stimulation	BC-EGF (82)
TGF-β (82)	Growth inhibition	
IGF-1 (83-86)	Androgen-stimulated growth/differentiation	
IL-1 (88)	Growth regulation	
Regulatory proteins		
Inhibin (93)	Androgen/androgen axis	
Müllerian duct inhibitory agent (94)	Anti-Sertoli cell development	
Sialoadhesin-2 (95, 96)	Sperm coating (96)	DAC-protein (93-98)
	Immunoconjugates (97, 98)	Clusters (99-101)
		245-253 (99)
		CM9-11 (100)
Metabolites		
Lactate/pyruvate (99-101)	Energy metabolism	
Exosome (102)	Signal transduction	

[Trends in Endocrinology and Metabolism](#)
[Volume 15, Issue 7](#), September 2004, Pages 345-350

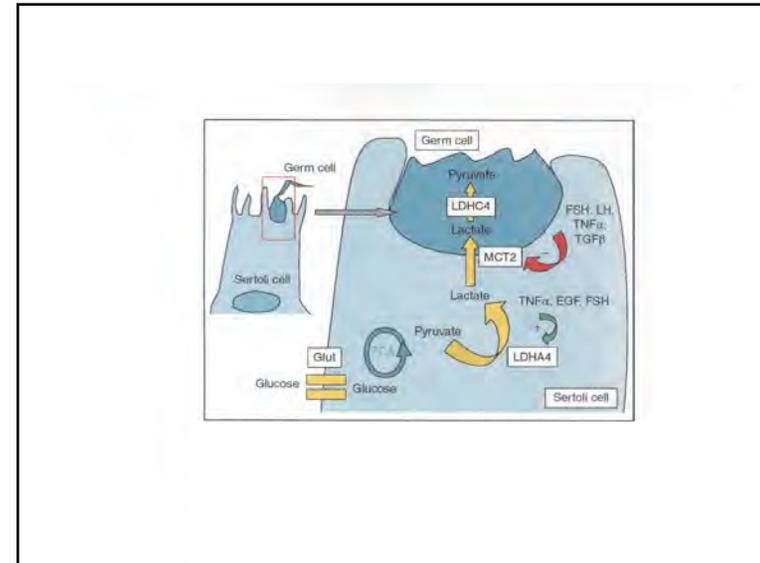
Lactate and energy metabolism in male germ cells

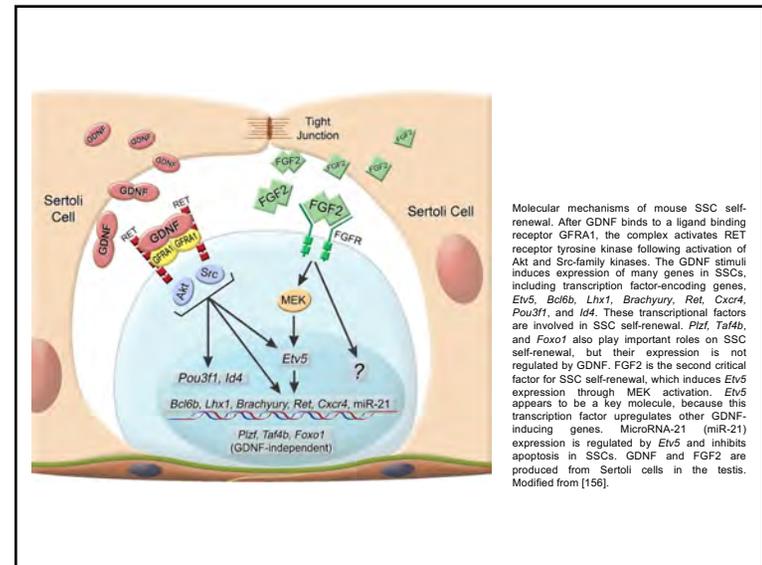
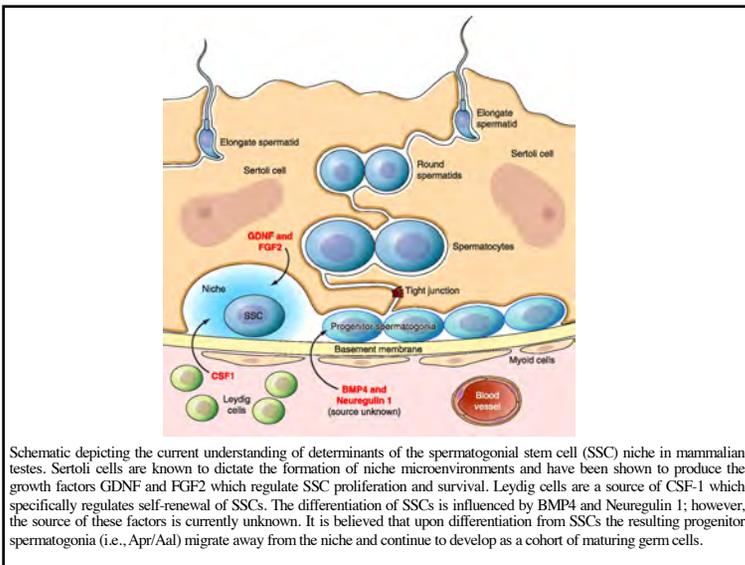
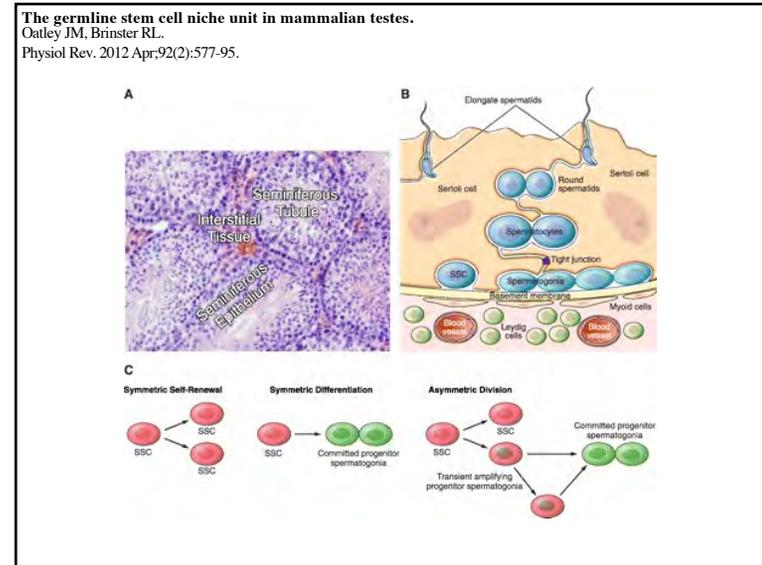
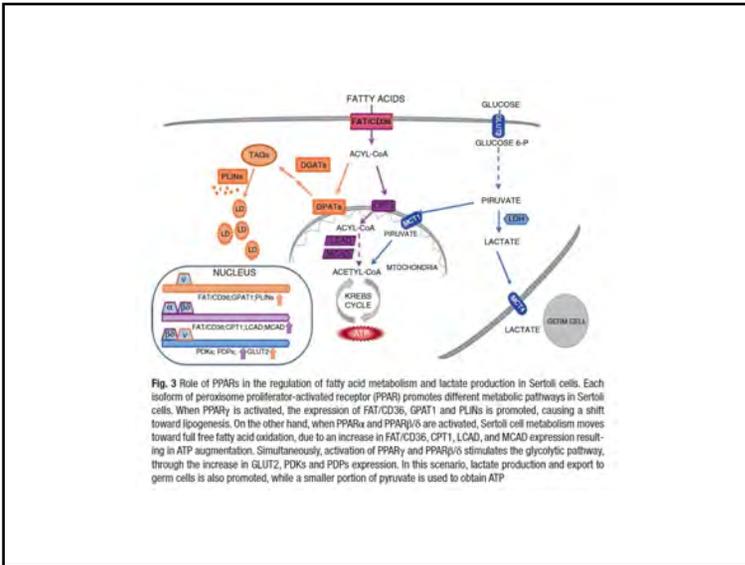
Fayçal Bousouar and Mohamed Benahmed

Inserm 407, Faculté de Médecine Lyon-Sud, 165 Chemin du Grand Revoyet, BP-12, F-69921 Oullins Cedex, France

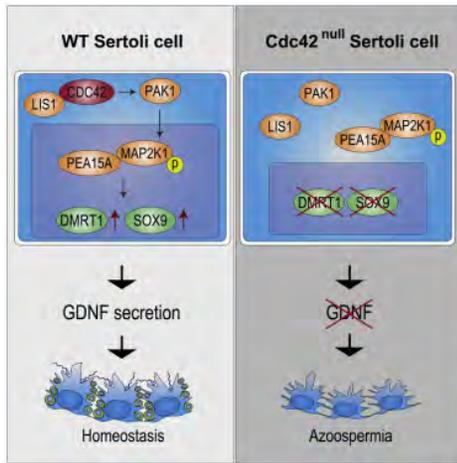
Available online 30 July 2004.

Various alterations in germ cell proliferation/differentiation, survival and energy metabolism are potentially involved in hypospermatogenesis leading to male infertility. Several reviews have been devoted to the different processes whose alteration might underlie hypospermatogenesis, except for energy metabolism in the testis. Energy metabolism in the testis exhibits some specificity in that lactate is the central energy metabolite used by germ cells. This metabolite is produced by somatic Sertoli cells, transported and used by germ cells in the context of an active cooperation under the control of the endocrine system and local cytokines. In this review, we present and discuss relevant published data on energy metabolism in male germ cells with a specific emphasis on lactate.

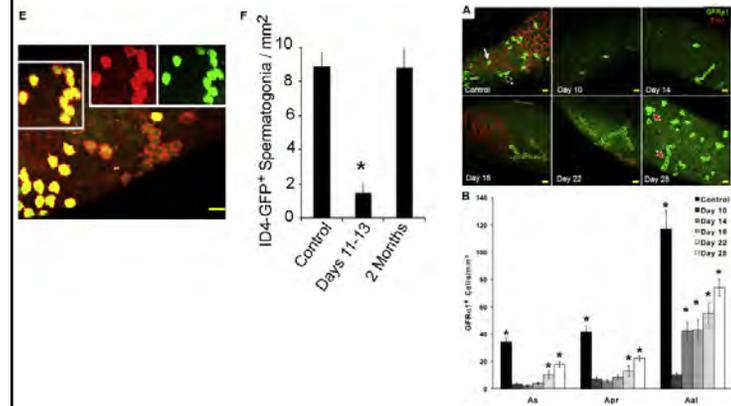




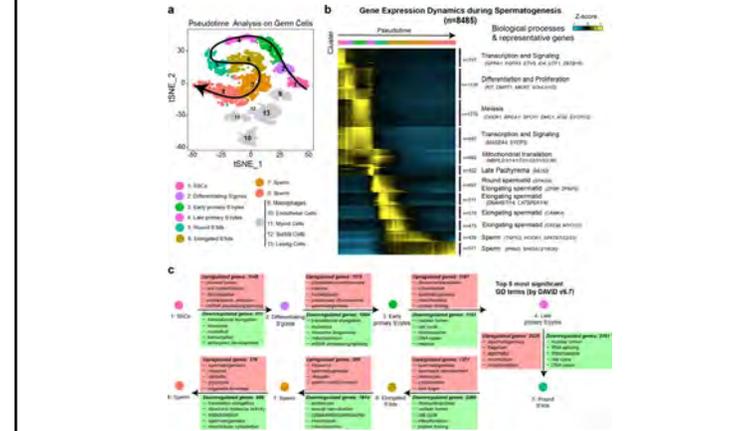
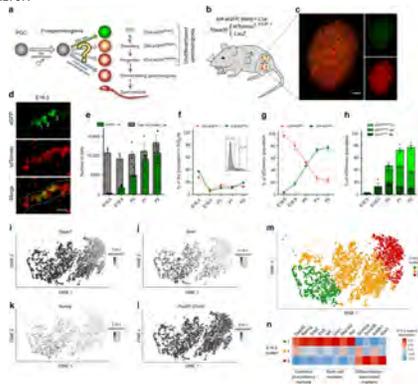
Cdc42 is required for male germline niche development in mice.
 Mori Y, Takashima S, Kanatsu-Shinohara M, et al.
 Cell Rep. 2021 Aug 17;36(7):109550.



Spermatogonial Stem Cell Numbers Are Reduced by Transient Inhibition of GDNF Signaling but Restored by Self-Renewing Replication when Signaling Resumes.
 Parker N, Laychur A, Sukwani M, et al.
 Stem Cell Reports. 2021 Mar 9;16(3):597-609.

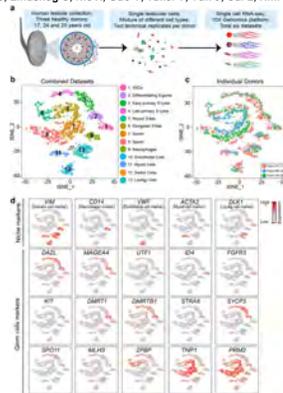


Developmental kinetics and transcriptome dynamics of stem cell specification in the spermatogenic lineage.
Law NC, Oatley MJ, Oatley JM.
Nat Commun. 2019 Jun 26;10(1):2787.



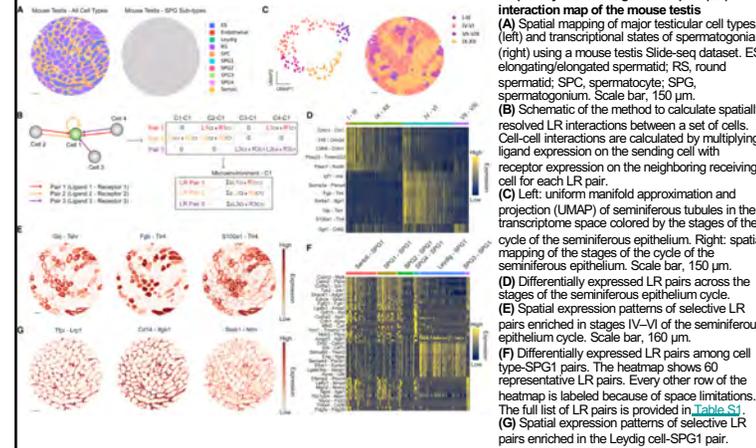
The adult human testis transcriptional cell atlas.

Guo J, Grow EJ, Micochova H, Maher GJ, Lindskog C, Nie X, Guo Y, Takei Y, Yun J, Cai L, Kim R, Carrell DT, Goriely A, Hotaling JM, Cairns BR.
Cell Res. 2018 Dec;28(12):1141-1157.



Dissecting the spermatogonial stem cell niche using spatial transcriptomics

Rajachandran S, Zhang X, Cao Q.
Cell Rep. 2023 Jul 25;42(7):112737.



A spatially resolved ligand-receptor (LR) interaction map of the mouse testis
(A) Spatial mapping of major testicular cell types (left) and transcriptional states of spermatogonia (right) using a mouse testis Slide-seq dataset. ES, elongating/elongated spermatid; RS, round spermatid; SPC, spermatocyte; SPG, spermatogonium. Scale bar, 150 μ m.
(B) Schematic of the method to calculate spatially resolved LR interactions between a set of cells. Cell-cell interactions are calculated by multiplying ligand expression on the sending cell with receptor expression on the neighboring receiving cell for each LR pair.
(C) Left: uniform manifold approximation and projection (UMAP) of seminiferous tubules in the transcriptome space colored by the stages of the cycle of the seminiferous epithelium. Right: spatial mapping of the stages of the cycle of the seminiferous epithelium. Scale bar, 150 μ m.
(D) Differentially expressed LR pairs across the stages of the seminiferous epithelium cycle.
(E) Spatial expression patterns of selective LR pairs enriched in stages IV-VI of the seminiferous epithelium cycle. Scale bar, 160 μ m.
(F) Differentially expressed LR pairs among cell type-SPG1 pairs. The heatmap shows 60 representative LR pairs. Every other row of the heatmap is labeled because of space limitations. The full list of LR pairs is provided in Table S1.
(G) Spatial expression patterns of selective LR pairs enriched in the Leydig cell-SPG1 pair.

Dissecting mammalian reproduction with spatial transcriptomics.
 Zhang X, Cao Q, Rajachandran S, Grow EJ, Evans M, Chen H.
 Hum Reprod Update. 2023 Nov 2;29(6):794-810.

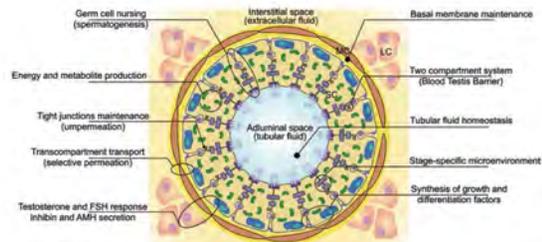
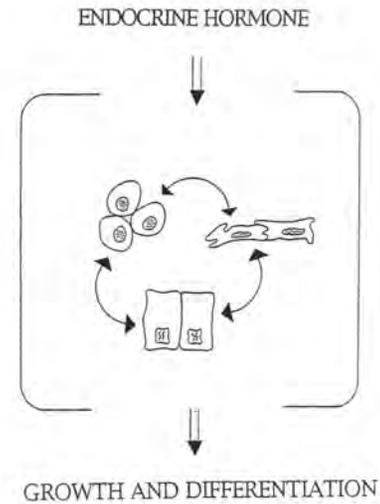
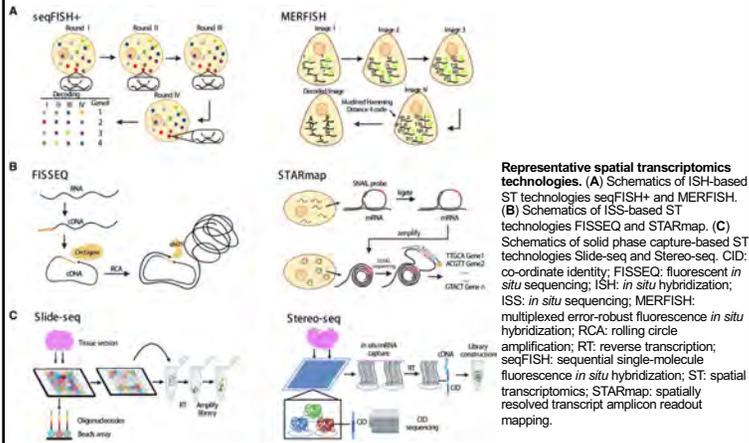
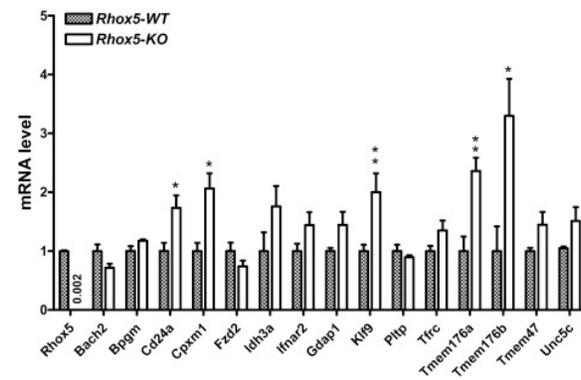
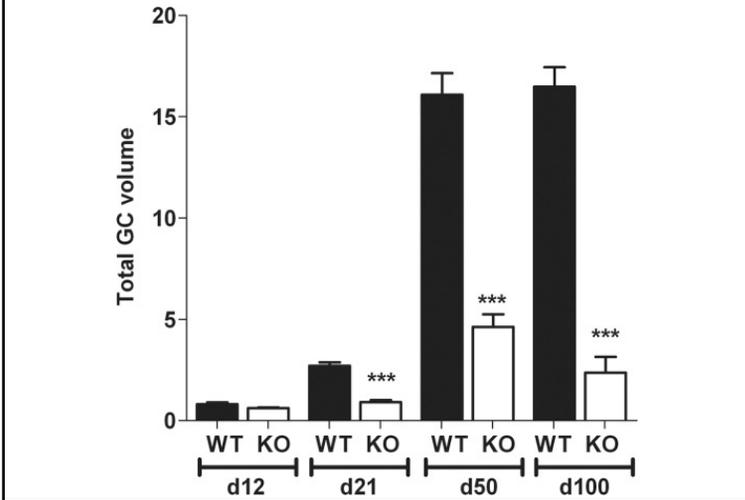


Fig. 1 The central role of Sertoli cells in testicular tissue environment and organization. Sertoli cells play several roles in testicular tissue's physiology. Besides their critical role on spermatogenesis, granting physical and nutritional support to developing germ cells, they also show paracrine and endocrine properties and are the main component of the blood-testis barrier, one of the tightest blood-tissue barriers of the human body

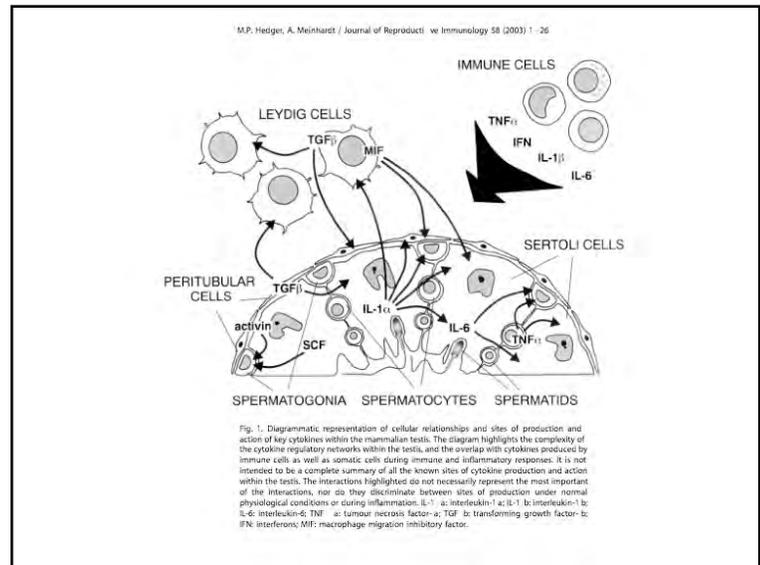
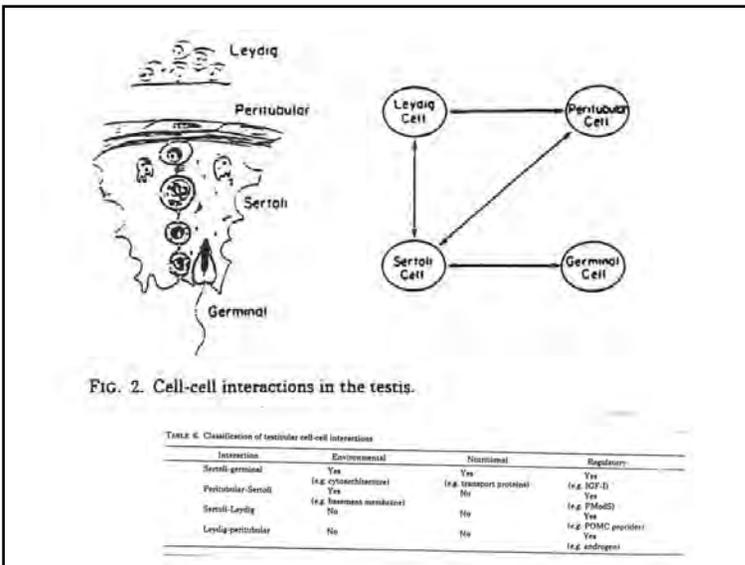
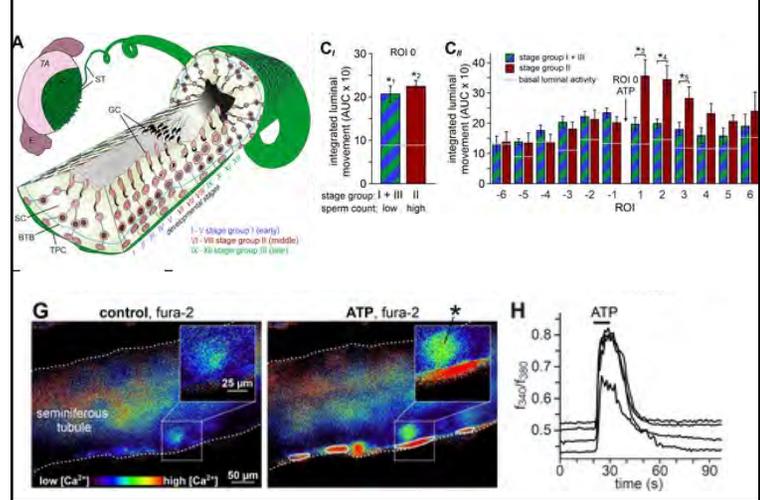
Androgen-induced Rhox homeobox genes modulate the expression of AR-regulated genes.
 Hu Z, Dandekar D, O'Shaughnessy PJ, De Gendt K, Verhoeven G, Wilkinson MF.
 Mol Endocrinol. 2010 Jan;24(1):60-75.



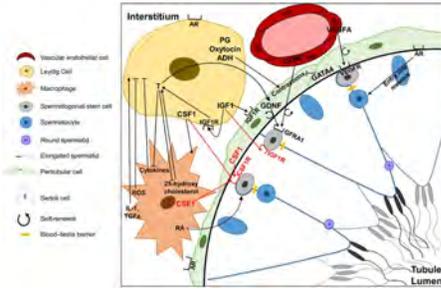
Androgen action via testicular peritubular myoid cells is essential for male fertility.
 Welsh M, Saunders PT, Atanassova N, Sharpe RM, Smith LB.
 FASEB J. 2009 Dec;23(12):4218-30.



ATP activation of peritubular cells drives testicular sperm transport.
 Fleck D, Kenzler L, Mundt N, et al.
 Elife. 2021 Jan 27;10:e62885.

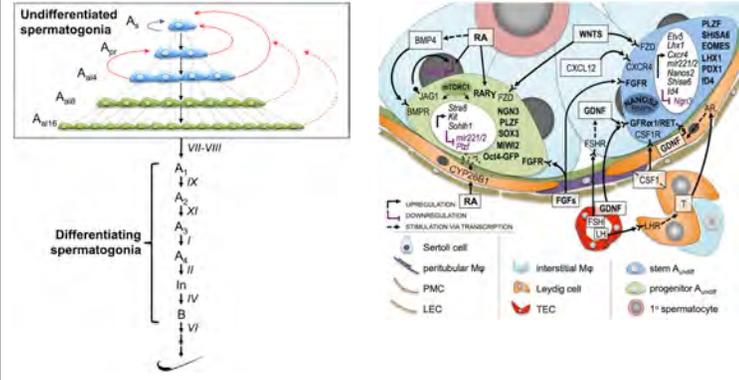


Essential roles of interstitial cells in testicular development and function.
 Heinrich A, DeFalco T.
 Andrology. 2019 Aug 24. doi: 10.1111/andr.12703.

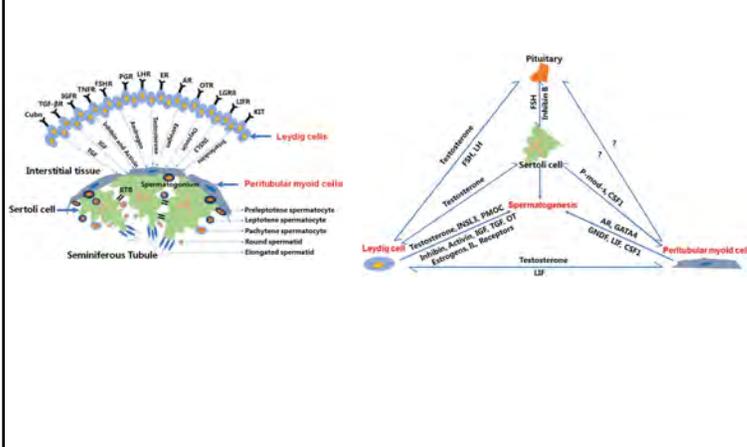


Role of interstitial cells in adult spermatogenesis. Illustration of a cross section of a seminiferous tubule and surrounding interstitium of a rodent testis, highlighting our current knowledge of the mechanisms through which testicular interstitial cells influence adult spermatogenesis. Text and receptors shown in red indicate interactions needing further study or are currently unclear. Arrows indicate a positive influence, and T-shaped lines indicate an inhibitory effect. ADH, vasopressin; AR, androgen receptor; CSF1/CSF1R, colony-stimulating factor 1/receptor; GDNF/GFRα1, glial cell-derived neurotrophic factor/receptor 1; IGF1/IGF1R, insulin-like growth factor 1/receptor; IL-1, interleukin-1; PG, prostaglandin; RA, retinoic acid; ROS, reactive oxygen species; T, testosterone; TGFα, transforming growth factor alpha; VEGFA/VEGFR, vascular endothelial growth factor/receptor.

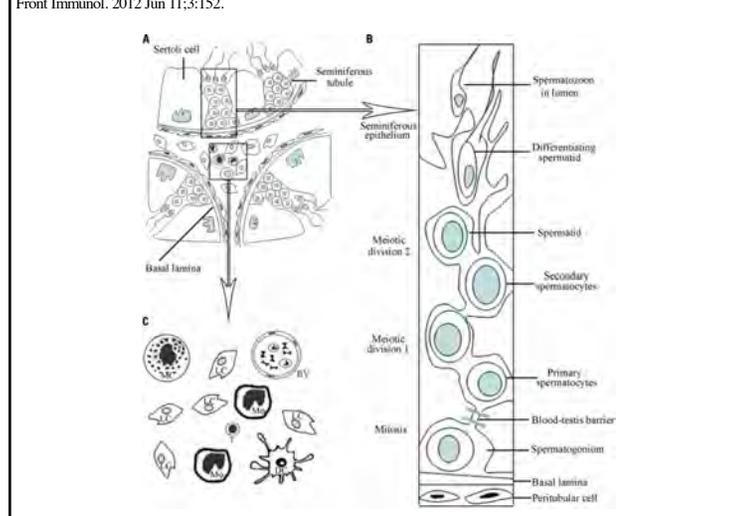
Molecular regulation of spermatogonial stem cell renewal and differentiation.
 Makelli JA, Hobbs RM.
 Reproduction. 2019 Jun 1. pii: REP-18-0476.R2.

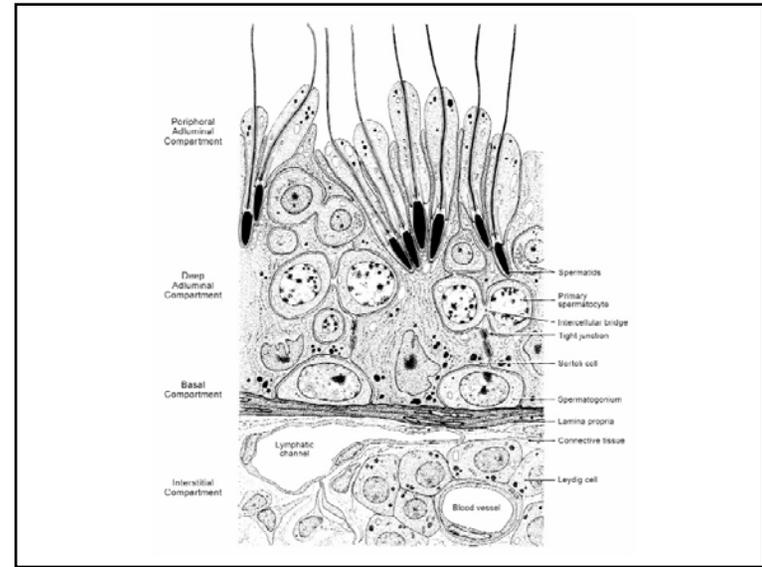
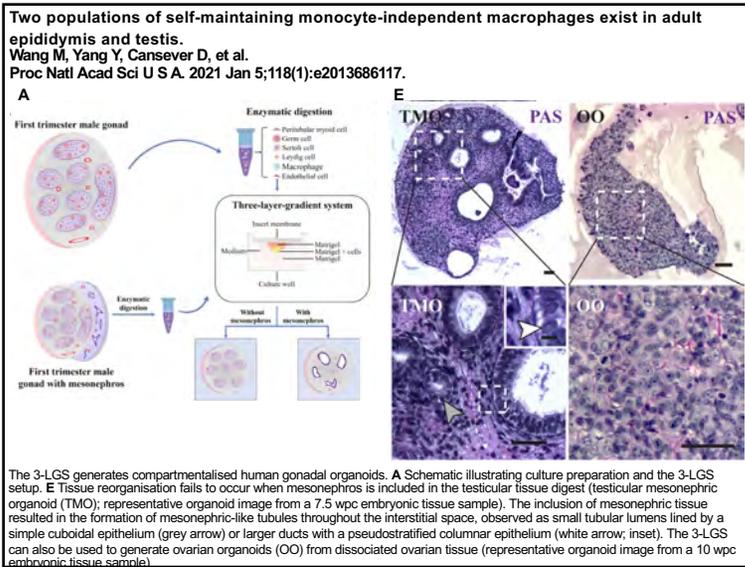
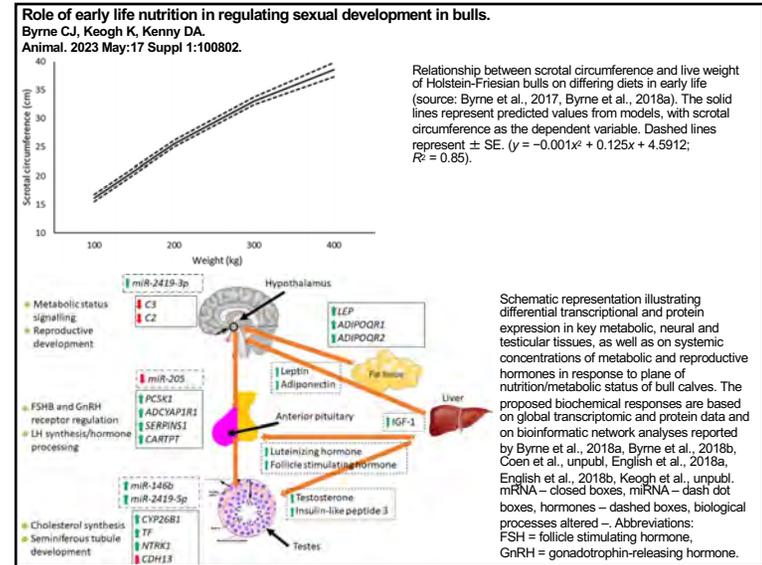
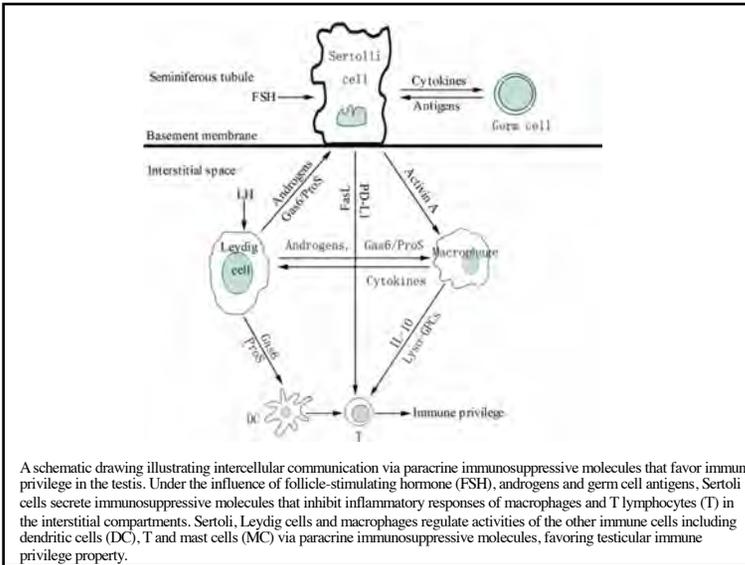


The roles and mechanisms of Leydig cells and myoid cells in regulating spermatogenesis.
 Zhou R, Wu J, Liu B, Jiang Y, Chen W, Li J, He Q, He Z.
 Cell Mol Life Sci. 2019 Jul;76(14):2681-2695.

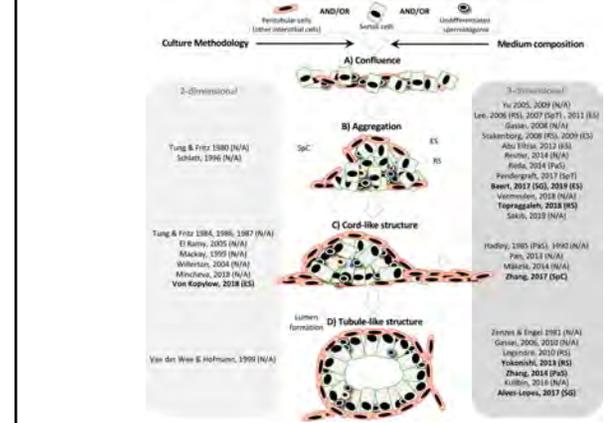
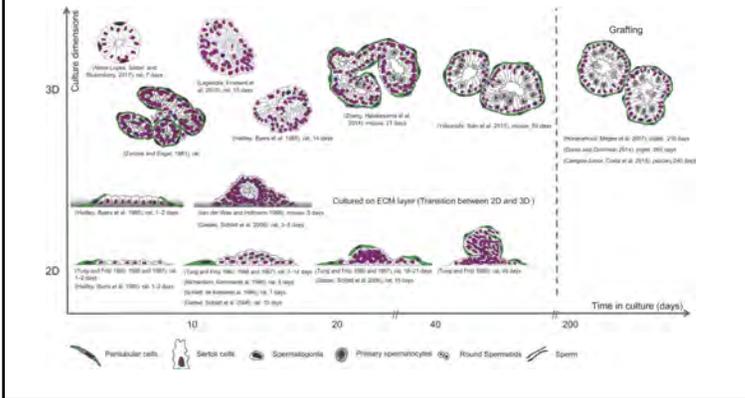


Structural, cellular and molecular aspects of immune privilege in the testis.
 Li N, Wang T, Han D.
 Front Immunol. 2012 Jun 11;3:152.

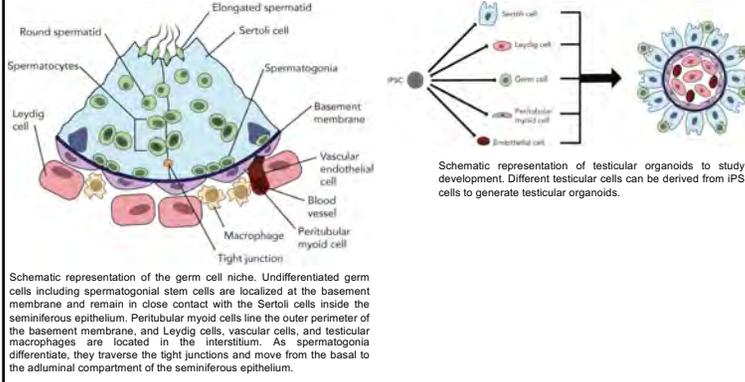




Testicular organoids: a new model to study the testicular microenvironment in vitro?
Hum Reprod Update. 2017 Dec 21. (Epub ahead of print)
Alves-Lopes JP, Stukenborg JB.

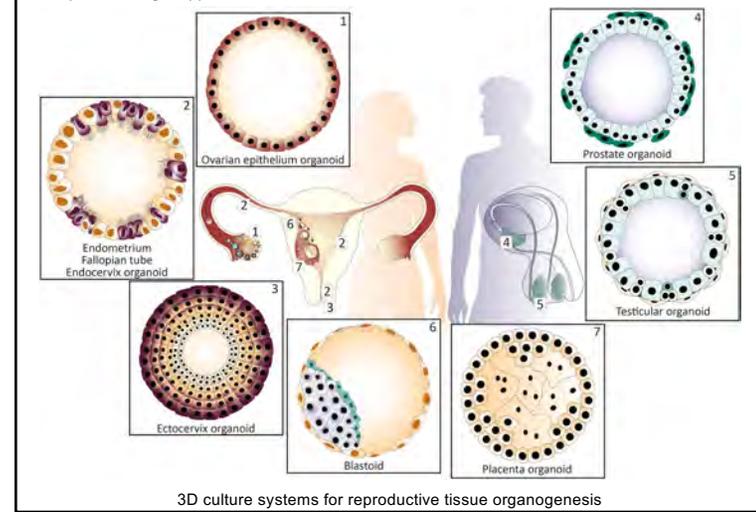


Testicular organoids to study cell-cell interactions in the mammalian testis.
Sakib S, Goldsmith T, Voigt A, Dobrinski I.
Andrology. 2019 Jul 21. doi: 10.1111/andr.12680.



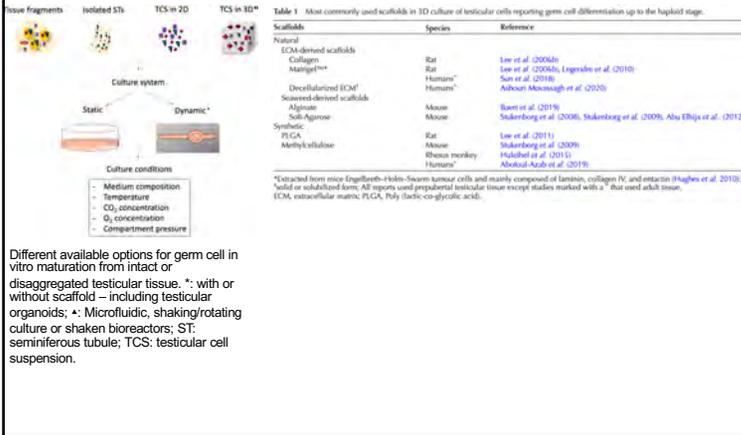
Schematic representation of the germ cell niche. Undifferentiated germ cells including spermatogonial stem cells are localized at the basement membrane and remain in close contact with the Sertoli cells inside the seminiferous epithelium. Peritubular myoid cells line the outer perimeter of the basement membrane, and Leydig cells, vascular cells, and testicular macrophages are located in the interstitium. As spermatogonia differentiate, they traverse the tight junctions and move from the basal to the adluminal compartment of the seminiferous epithelium.

Human organoid systems in modeling reproductive tissue development, function, and disease.
Haider S, Beristain AG.
Hum Reprod. 2023 Aug 1;38(8):1449-1463.



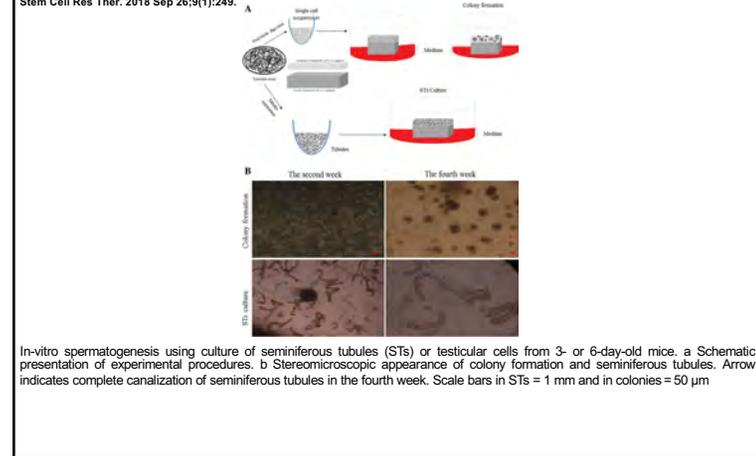
3D culture systems for reproductive tissue organogenesis

Organoids as tools to investigate the molecular mechanisms of male infertility and its treatments
 Kanbar M, Vermeulen M, Wyns C.
 Reproduction. 2021 May;161(5):R103-R112.



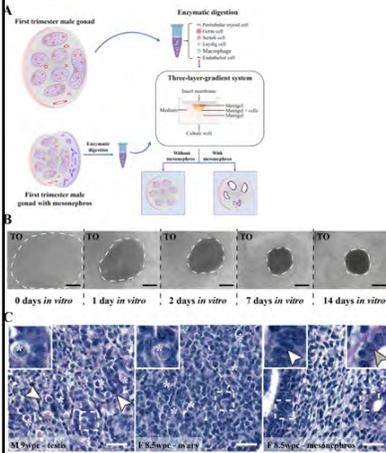
Different available options for germ cell in vitro maturation from intact or disaggregated testicular tissue. †: with or without scaffold – including testicular organoids; ‡: Microfluidic, shaking/rotating culture or shaken bioreactors; ST: seminiferous tubule; TCS: testicular cell suspension.

Organ culture of seminiferous tubules using a modified soft agar culture system.
 Gholami K, Pourmand G, Koruji M, Ashouri S, Abbasi M.
 Stem Cell Res Ther. 2018 Sep 26;9(1):249.

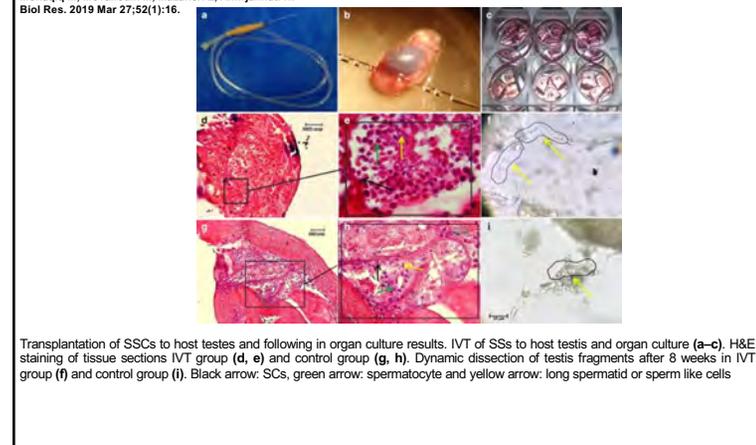


Self-organising human gonads generated by a Matrigel-based gradient system.

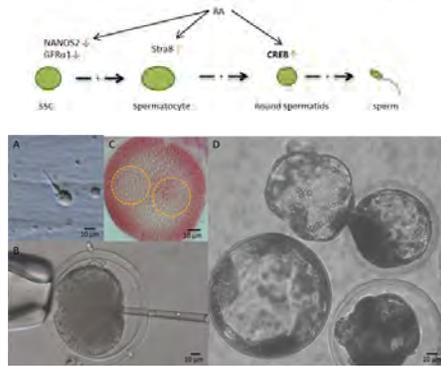
Oliver E, Alves-Lopes JP, Femke Hartevel F, et al.
 BMC Biol. 2021 Sep 23;19(1):212.



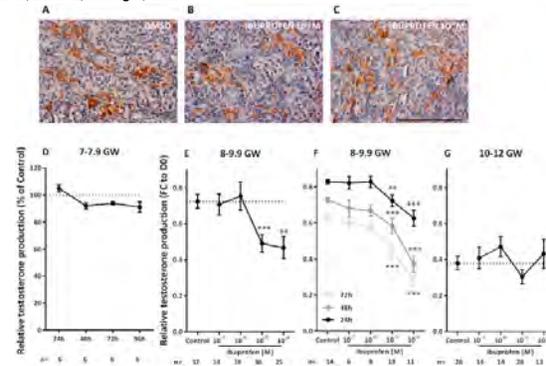
In vitro transplantation of spermatogonial stem cells isolated from human frozen-thawed testis tissue can induce spermatogenesis under 3-dimensional tissue culture conditions.
 Mohaqiq M, Movahedin M, Mazaheri Z, Amirjannati N.
 Biol Res. 2019 Mar 27;52(1):16.



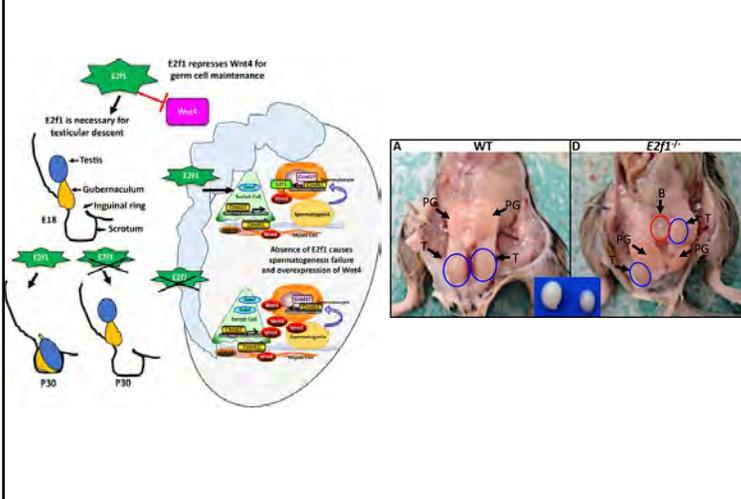
In-vitro differentiation of early pig spermatogenic cells to haploid germ cells.
 Yu K, Zhang Y, Zhang BL, Wu HY, Jiang WQ, Wang ST, Han DP, Liu YX, Lian ZX, Deng SL.
Mol Hum Reprod. 2019 Sep 1;25(9):507-516.



Ibuprofen results in alterations of human fetal testis development
 Sci Rep. 2017 Mar 10;7:44184.
 Ben Maamar M, Lesné L, Hennig K, et al.



E2F1 regulates testicular descent and controls spermatogenesis by influencing WNT4 signaling
 Jorgez CJ, Seth A, Wilken N, et al.
Development. 2021 Jan 13;148(1):dev191189.



Genetic dissection of spermatogenic arrest through exome analysis: clinical implications for the management of azoospermic men.
 Krausz C, Riera-Escamilla A, Moreno-Mendoza D, et al.
Genet Med. 2020 Dec;22(12):1956-1966.

Abstract

Purpose: Azoospermia affects 1% of men and it can be the consequence of spermatogenic maturation arrest (MA). Although the etiology of MA is likely to be of genetic origin, only 13 genes have been reported as recurrent potential causes of MA.

Methods: Exome sequencing in 147 selected MA patients (discovery cohort and two validation cohorts).

Results: We found strong evidence for five novel genes likely responsible for MA (ADAD2, TERB1, SHOC1, MSH4, and RAD21L1), for which mouse knockout (KO) models are concordant with the human phenotype. Four of them were validated in the two independent MA cohorts. In addition, nine patients carried pathogenic variants in seven previously reported genes-TEX14, DMRT1, TEX11, SYCE1, MEIOB, MEI1, and STAG3-allowing to upgrade the clinical significance of these genes for diagnostic purposes. Our meiotic studies provide novel insight into the functional consequences of the variants, supporting their pathogenic role.

Conclusion: Our findings contribute substantially to the development of a pre-testicular sperm extraction (TESE) prognostic gene panel. If properly validated, the genetic diagnosis of complete MA prior to surgical interventions is clinically relevant. Wider implications include the understanding of potential genetic links between nonobstructive azoospermia (NOA) and cancer predisposition, and between NOA and premature ovarian failure.

"Systems Biology of Reproduction"

Spring 2024 (Even Years) – Course Syllabus

Biol 475/575 Undergraduate/Graduate (3 Credit)

SLN: (475) – 06763, (575) – 06764

Time - Tuesday and Thursday 10:35 am-11:50 am

Course Lectures in person and recorded on Canvas/Panopto and Discussion Sessions live in person and on WSU Zoom for all campuses (Hybrid Course)

Room – CUE 418

Course Director – Michael Skinner, Abelson Hall 507, 335-1524, skinner@wsu.edu

Co-Instructor – Eric Nilsson, Abelson Hall 507, 225-1835, nilsson@wsu.edu

Learning Objective -

Current literature based course on the Systems Biology of Reproduction. Learning Systems approaches to the biology of reproduction from a molecular to physiological level of understanding.

Schedule/Lecture Outline –

January	9 & 11	Week 1	Systems Biology Introduction
	16 & 18	Week 2	Molecular/ Cellular/ Reproduction Systems
	23 & 25	Week 3	Sex Determination Systems
Jan /Feb	30 & 1	Week 4	Male Reproductive Tract Development & Function
February	6 & 8	Week 5	Female Reproductive Tract Development & Function
	13 & 15	Week 6	Gonadal Developmental Systems Biology
	20 & 22	Week 7	Testis Systems Biology
	27 & 29	Week 8	Ovary Systems Biology
March	5 & 7	Week 9	Epigenetics and Transgenerational Gonadal Disease
	11 – 15	Week 10	Spring Break
	19 & 21	Week 11	Gametogenesis/ Stem Cells/ Cloning
	26 & 28	Week 12	Hypothalamus- Pituitary Development & Function
April	2 & 4	Week 13	Reproductive Endocrinology Systems
	9 & 11	Week 14	Fertilization & Implantation Systems
	16 & 18	Week 15	Fetal Development & Birth Systems
	23 & 25	Week 16	Assisted Reproduction/Contraception
Apr/May	30 & 2	Week 17	Exam or Grant Review