Spring 2024 – Systems Biology of Reproduction
Lecture Outline – Fertilization & Implantation Systems
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
April 9, 2024
Week 14

Fertilization & Implantation Systems

Fertilization –
- Sperm and female reproductive tract
- Attraction, hyperactivation, binding, acrosome reaction
- Penetration, sperm-egg fusion
- PLC and calcium mobilization
- Fertilization and embryo induction

Implantation –
- Embryo development and fallopian tube
- Endocrine induction of uterine development
- Uterine cell biology, vascularization and maturation
- Proliferative and secretory stage
- Blastula and endometrium interactions
- Implantation apposition, adhesion, invasion and system biology

Required Reading

Evans JP. (2018) Fertilization in the Oviduct. In: Encyclopedia of Reproduction 2nd Edition, Ed: MK Skinner. Elsevier. Vol 3:300-304.

Deguchi R, Hirohashi N. (2018) Fertilization, Comparative. In: Encyclopedia of Reproduction 2nd Edition, Ed: MK Skinner. Elsevier. Vol 6:344-349.

Cha JM, et al. (2018) Aspects of Rodent Implantation. In: Encyclopedia of Reproduction 2nd Edition, Ed: MK Skinner. Elsevier. Vol 2:291-297.

Lu J, Kong S, Wang H. (2018) Uterine Receptivity: The Status of Uterus for Implantation. In: Encyclopedia of Reproduction 2nd Edition, Ed: MK Skinner. Elsevier. Vol 2:394-399.

REFERENCES

Lu Y, Shimada K, Tang S, et al. 1700029I15Rik orchestrates the biosynthesis of acrosomal membrane proteins required for sperm-egg interaction. Proc Natl Acad Sci U S A. 2023 Feb 21;120(8):e2207263120.

Kanatsu-Shinohara M, Shiromoto Y, Ogonuki N, et al. Intracytoplasmic sperm injection induces transgenerational abnormalities in mice. J Clin Invest. 2023 Nov 15;133(22):e170140.
Wolfner MF, Suarez SS, Dorus S. Suspension of hostility: Positive interactions between spermatozoa and female reproductive tracts. Andrology. 2023 Jul;11(5):943-947.
Liu L, Oura S, Markham Z, Hamilton JN, et al. Modeling post-implantation stages of human development into early organogenesis with stem-cell-derived peri-gastruloids. Cell. 2023 Aug 31;186(18):3776-3792.e16.
Hemberger M, Dean W. The placenta: epigenetic insights into trophoblast developmental models of a generation-bridging organ with long-lasting impact on lifelong health. Physiol Rev. 2023 Oct 1;103(4):2523-2560.
Yao H, Sun N, Shao H, Wang T, Tan T. Ex utero embryogenesis of non-human primate embryos and beyond. Curr Opin Genet Dev. 2023 Oct;82:102093.
Regin M, Essahib W, Demtschenko A, et al. Lineage segregation in human pre-implantation embryos is specified by YAP1 and TEAD1. Hum Reprod. 2023 Aug 1;38(8):1484-1498.
Butt Z, Tinning H, O'Connell MJ, Fenn J, Alberio R, Forde N. Understanding conceptus-maternal interactions: what tools do we need to develop? Reprod Fertil Dev. 2023 Dec;36(2):81-92.
Saouli A, Adjroud O, Ncir M, Bachir A, El Feki A. Attenuating effects of selenium and zinc against hexavalent chromium-induced oxidative stress, hormonal instability, and placenta damage in preimplanted rats. Environ Sci Pollut Res Int. 2023 May;30(21):60050-60079.
Rodriguez-Martinez H, Martinez EA, Calvete JJ, Peña Vega FJ, Roca J. Seminal Plasma: Relevant for Fertility? Int J Mol Sci. 2021 Apr 22;22(9):4368.
Kobayashi W, Tachibana K. Awakening of the zygotic genome by pioneer transcription factors. Curr Opin Struct Biol. 2021 Dec;71:94-100.
Escobar Carreiro LE, Dos Santos GS, Luedke FE, Goissis MD. Cell differentiation events in pre-implantation mouse and bovine embryos. Anim Reprod. 2022 Jan 7;18(4):e20210054.
Zhao Y, Vanderkooi S, Kan FWK. The role of oviduct-specific glycoprotein (OVGP1) in modulating biological functions of gametes and embryos. Histochem Cell Biol. 2022 Jan 6. doi: 10.1007/s00418-021-02065-x. Online ahead of print.
Stäubli A, Peters AH. Mechanisms of maternal intergenerational epigenetic inheritance. Curr Opin Genet Dev. 2021 Apr;67:151-162.
Paonessa M, Borini A, Coticchio G. Genetic causes of preimplantation embryo developmental failure. Mol Reprod Dev. 2021 May;88(5):338-348.
Kolanska K, Bendifallah S, Canlorbe G, et al. Role of miRNAs in Normal Endometrium and in Endometrial Disorders: Comprehensive Review. J Clin Med. 2021 Aug 4;10(16):3457.
Nilsson LL, Hviid TVF. HLA Class Ib-receptor interactions during embryo implantation and early pregnancy. Hum Reprod Update. 2022 Mar 2;dmac007. doi: 10.1093/humupd/dmac007. Online ahead of print.

Luddi A, Pavone V, Governini L, et al. Emerging role of embryo secretome in the paracrine communication at the implantation site: a proof of concept. Fertil Steril. 2021 Apr;115(4):1054-1062.
Kim MJ, Kim YS, Kim YJ, et al. Upregulation of Low-Density Lipoprotein Receptor of the Steroidogenesis Pathway in the Cumulus Cells Is Associated with the Maturation of Oocytes and Achievement of Pregnancy. Cells. 2021 Sep 11;10(9):2389.
Hwang JY, Maziarz J, Wagner GP, Chung JJ. Molecular Evolution of CatSper in Mammals and Function of Sperm Hyperactivation in Gray Short-Tailed Opossum. Cells. 2021 Apr 29;10(5):1047.
Yang F, Gracia Gervasi M, Orta G, et al. C2CD6 regulates targeting and organization of the CatSper calcium channel complex in sperm flagella. Development. 2022 Jan 15;149(2):dev199988.
Giaccagli MM, Gómez-Elías MD, Herzfeld JD, et al. Capacitation-Induced Mitochondrial Activity Is Required for Sperm Fertilizing Ability in Mice by Modulating Hyperactivation. Front Cell Dev Biol. 2021 Oct 26;9:767161.
Hwang JY, Wang H, Lu Y, et al. C2cd6-encoded CatSperτ targets sperm calcium channel to Ca 2+ signaling domains in the flagellar membrane. Cell Rep. 2022 Jan 18;38(3):110226.
Saxena DK, Toshimori K. Molecular modifications of MC31/CE9, a sperm surface molecule, during sperm capacitation and the acrosome reaction in the rat: is MC31/CE9 required for fertilization? Biol Reprod. 2004 Apr;70(4):993-1000.
Aldana A, Carneiro J, Martínez-Mekler G, Darszon A. Discrete Dynamic Model of the Mammalian Sperm Acrosome Reaction: The Influence of Acrosomal pH and Physiological Heterogeneity. Front Physiol. 2021 Jul 19;12:682790.
Siu KK, Serrão VHB, Ziyyat A, Lee JE. The cell biology of fertilization: Gamete attachment and fusion. J Cell Biol. 2021 Oct 4;220(10):e202102146.
Carrasquel Martínez G, Aldana A, Carneiro J, et al. Acrosomal alkalinization occurs during human sperm capacitation. Mol Hum Reprod. 2022 Mar 8;28(3):gaac005.
Mata-Martínez E, Sánchez-Tusie AA, Darszon A, et al. Epac activation induces an extracellular Ca 2+ -independent Ca 2+ wave that triggers acrosome reaction in human spermatozoa. Andrology. 2021 Jul;9(4):1227-1241.
Birch MR, Dissing S, Skakkebæk NE, Rehfeld A. Finasteride interferes with prostaglandin-induced CatSper signalling in human sperm. Reproduction. 2021 May;161(5):561-572.
Carvalho RK, Rocha TL, Fernandes FH, et al. Decreasing sperm quality in mice subjected to chronic cannabidiol exposure: New insights of cannabidiol-mediated male reproductive toxicity. Chem Biol Interact. 2022 Jan 5;351:109743.
Inoue N, Hagihara Y, Wada I. Evolutionarily conserved sperm factors, DCST1 and DCST2, are required for gamete fusion. Elife. 2021 Apr 19;10:e66313.
Deneke VE, Pauli A. The Fertilization Enigma: How Sperm and Egg Fuse. Annu Rev Cell Dev Biol. 2021 Oct 6;37:391-414.
Binner MI, Kogan A, Panser K, et al. The Sperm Protein Spaca6 is Essential for Fertilization in Zebrafish. Front Cell Dev Biol. 2022 Jan 3;9:806982.
Wessel GM, Wada Y, Yajima M, Kiyomoto M. Sperm lacking Bindin are infertile but are otherwise indistinguishable from wildtype sperm. Sci Rep. 2021 Nov 3;11(1):21583.

Takei GL, Tourzani DA, Paudel B, Visconti PE. Activation of cAMP-dependent phosphorylation pathways is independent of ROS production during mouse sperm capacitation. Mol Reprod Dev. 2021 Aug;88(8):544-557.
Khan HL, Bhatti S, Abbas S, et al. Extracellular microRNAs: key players to explore the outcomes of in vitro fertilization. Reprod Biol Endocrinol. 2021 May 15;19(1):72.
Carlisle JA, Swanson WJ. Molecular mechanisms and evolution of fertilization proteins. J Exp Zool B Mol Dev Evol. 2021 Dec;336(8):652-665.
Governini L, Luongo FP, Haxhiu A, et al. Main actors behind the endometrial receptivity and successful implantation. Tissue Cell. 2021 Dec;73:101656.
Maurya VK, DeMayo FJ, Lydon JP. Illuminating the "Black Box" of Progesterone-Dependent Embryo Implantation Using Engineered Mice. Front Cell Dev Biol. 2021 Apr 7;9:640907.
Tomoda K, Hu H, Sahara Y, et al. Reprogramming epiblast stem cells into pre-implantation blastocyst cell-like cells. Stem Cell Reports. 2021 May 11;16(5):1197-1209.
Hajipour H, Farzadi L, Roshangar L, et al. A human chorionic gonadotropin (hCG) delivery platform using engineered uterine exosomes to improve endometrial receptivity. Life Sci. 2021 Jun 15;275:119351.
Sebastian-Leon P, Devesa-Peiro A, Aleman A, et al. Transcriptional changes through menstrual cycle reveal a global transcriptional derepression underlying the molecular mechanism involved in the window of implantation. Mol Hum Reprod. 2021 May 8;27(5):gaab027.
Anamthathmakula P, Winuthayanon W. Prostaglandin-Endoperoxide Synthase 2 (PTGS2) in the Oviduct: Roles in Fertilization and Early Embryo Development. Endocrinology. 2021 Apr 1;162(4):bqab025.
Cai W, Yang L, Zhang R, Yang Y, et al. Abnormally increased DNA methylation in chorionic tissue might play an important role in development of ectopic pregnancy. Reprod Biol Endocrinol. 2021 Jul 2;19(1):101.
Jiang H, Li JX. Interaction networks between the Fallopian tubes and the embryo in human tubal pregnancy: Current knowledge and perspectives. J Obstet Gynaecol Res. 2021 Dec;47(12):4139-4147.
McGlade EA, Herrera GG, Stephens KK, et al. Cell-type specific analysis of physiological action of estrogen in mouse oviducts. FASEB J. 2021 May;35(5):e21563.
Silva ESD, Amaral C, Barreta M, et al. FGF18 modulates CTGF mRNA expression in cumulus-oocyte complexes and early bovine embryos: preliminary data. Zygote. 2021 Aug 18;1-5. doi: 10.1017/S0967199421000599. Online ahead of print.
Stewart CA, Stewart MD, Wang Y, et al. Chronic Estrus Disrupts Uterine Gland Development and Homeostasis. Endocrinology. 2022 Mar 1;163(3):bqac011.
Stenhouse C, Seo H, Wu G, et al. Insights into the Regulation of Implantation and Placentation in Humans, Rodents, Sheep, and Pigs. Adv Exp Med Biol. 2022;1354:25-48.
Kramer AC, Erikson DE, McLendon BA, et al. SPP1 expression in the mouse uterus and placenta: implications for implantation† Biol Reprod. 2021 Oct 11;105(4):892-904.

Bienert M, Habib P, Buck V, et al. Intrauterine hCG application increases expression of endothelial cell-cell adhesion molecules in human. Arch Gynecol Obstet. 2021 Dec;304(6):1587-1597.
Mahdavinezhad F, Gharaei R, Farmani AR, Hashemi F, Kouhestani M, Amidi F. The Potential Relationship Between Different Human Female Reproductive Disorders and Sperm Quality in Female Genital Tract. Reprod Sci. 2022 Mar;29(3):695-710.
Gòdia M, Reverter A, González-Prendes R, Ramayo-Caldas Y, Castelló A, Rodríguez-Gil JE, Sánchez A, Clop A. A systems biology framework integrating GWAS and RNA-seq to shed light on the molecular basis of sperm quality in swine. Genet Sel Evol. 2020 Dec 8;52(1):72.
Berta DG, Kuisma H, Välimäki N, et al. Deficient H2A.Z deposition is associated with genesis of uterine leiomyoma. Nature. 2021 Aug;596(7872):398-403.
Groff AF, Resetkova N, DiDomenico F, et al. RNA-seq as a tool for evaluating human embryo competence. Genome Res. 2019 Oct;29(10):1705-1718.
Trötschel C, Hamzeh H, Alvarez L, et al. Absolute proteomic quantification reveals design principles of sperm flagellar chemosensation. EMBO J. 2020 Feb 17;39(4):e102723.
Liu S, Fang L, Zhou Y, Santos DJA, et al. Analyses of inter-individual variations of sperm DNA methylation and their potential implications in cattle. BMC Genomics. 2019 Nov 21;20(1):888.
Chan MM, Smith ZD, Grosswendt S, et al. Molecular recording of mammalian embryogenesis. Nature. 2019 Jun;570(7759):77-82.
Skinner WM, Mannowetz N, Lishko PV, Roan NR. Single-cell Motility Analysis of Tethered Human Spermatozoa. Bio Protoc. 2019 Mar 5;9(5).
Smith HL, Stevens A, Minogue B, Sneddon S, et al. Systems based analysis of human embryos and gene networks involved in cell lineage allocation. BMC Genomics. 2019 Mar 5;20(1):171.
Engel KM, Baumann S, Rolle-Kampczyk U, et al. Metabolomic profiling reveals correlations between spermiogram parameters and the metabolites present in human spermatozoa and seminal plasma. PLoS One. 2019 Feb 20;14(2):e0211679.
Taei A, Rasooli P, Braun T, Hassani SN, Baharvand H. Signal regulators of human naïve pluripotency. Exp Cell Res. 2020 Feb 26:111924.
Da Broi MG, Meola J, Plaça JR, Peronni KC, et al. Is the profile of transcripts altered in the eutopic endometrium of infertile women with endometriosis during the implantation window? Hum Reprod. 2019 Dec 1;34(12):2381-2390.
Fan H, Jiang L, Lee YL, Wong CKC, Ng EHY, Yeung WSB, Lee KF. Bisphenol compounds regulate decidualized stromal cells in modulating trophoblastic spheroid outgrowth and invasion in vitro†. Biol Reprod. 2020 Mar 13;102(3):693-704.
Niwayama R, Moghe P, Liu YJ, Fabrèges D, et al. A Tug-of-War between Cell Shape and Polarity Controls Division Orientation to Ensure Robust Patterning in the Mouse Blastocyst. Dev Cell. 2019 Dec 2;51(5):564-574.e6.
Tan L, Lacko L, Zhou T, et al. Pre- and peri-implantation Zika virus infection impairs fetal development by targeting trophectoderm cells. Nat Commun. 2019 Sep 13;10(1):4155.

Wilsterman K, Bao X, Estrada AD, Comizzoli P, Bentley GE. Sex steroids influence organizational but not functional decidualization of feline endometrial cells in a 3D culture system†. Biol Reprod. 2019 Nov 21;101(5):906-915.
Fooladi H, Moradi P, Sharifi-Zarchi A, Hosein Khalaj B. Enhanced Waddington landscape model with cell-cell communication can explain molecular mechanisms of self-organization. Bioinformatics. 2019 Oct 15;35(20):4081-4088.
Wang X, Li X, Wang T, Wu SP, Jeong JW, et al. SOX17 regulates uterine epithelial-stromal cross-talk acting via a distal enhancer upstream of Ihh. Nat Commun. 2018 Oct 24;9(1):4421.
Rytkönen KT, Erkenbrack EM, Poutanen M, et al. Decidualization of Human Endometrial Stromal Fibroblasts is a Multiphasic Process Involving Distinct Transcriptional Programs. Reprod Sci. 2019 Mar;26(3):323-336.
Vilarino M, Suchy FP, Rashid ST, Lindsay H, et al. Mosaicism diminishes the value of pre-implantation embryo biopsies for detecting CRISPR/Cas9 induced mutations in sheep. Transgenic Res. 2018 Dec;27(6):525-537.
Sano S, Oshima K, Wang Y, Katanasaka Y, Sano M, Walsh K. CRISPR-Mediated Gene Editing to Assess the Roles of Tet2 and Dnmt3a in Clonal Hematopoiesis and Cardiovascular Disease. Circ Res. 2018 Jul 20;123(3):335-341.
Lee HJ, Choi NY, Park YS, Lee S, Bang JS, et al. Multigenerational effects of maternal cigarette smoke exposure during pregnancy on sperm counts of F1 and F2 male offspring. Reprod Toxicol. 2018 Jun;78:169-177.
Mathyk B, Adams N, Young SL. Endometrial receptivity: lessons from systems biology and candidate gene studies of endometriosis. Minerva Ginecol. 2017 Feb;69(1):41-56.
Sjunnesson Y. In vitro fertilisation in domestic mammals-a brief overview. Ups J Med Sci. 2019 Dec 13:1-9. doi: 10.1080/03009734.2019.1697911. [Epub ahead of print]
Telfer EE. Future developments: In vitro growth (IVG) of human ovarian follicles. Acta Obstet Gynecol Scand. 2019 May;98(5):653-658.
Adams NL, Heyland A, Rice LL, Foltz KR. Procuring animals and culturing of eggs and embryos. Methods Cell Biol. 2019;150:3-46
Miller DJ. Review: The epic journey of sperm through the female reproductive tract. Animal. 2018 Jun;12(s1):s110-s120.
Suarez SS. Mammalian sperm interactions with the female reproductive tract. Cell Tissue Res. 2016 Jan;363(1):185-194.
Rickard JP, de Graaf SP. Sperm surface changes and their consequences for sperm transit through the female reproductive tract. Theriogenology. 2020 Feb 10. pii: S0093-691X(20)30120-5. doi: 10.1016/j.theriogenology.2020.02.018. [Epub ahead of print]
Pitnick S, Wolfner MF, Dorus S. Post-ejaculatory modifications to sperm (PEMS). Biol Rev Camb Philos Soc. 2020 Apr;95(2):365-392.
Noda T, Ikawa M. Physiological function of seminal vesicle secretions on male fecundity. Reprod Med Biol. 2019 Jun 17;18(3):241-246.
Morgan HL, Watkins AJ. The influence of seminal plasma on offspring development and health. Semin Cell Dev Biol. 2020 Jan;97:131-137.
Rickard JP, Pool KR, Druart X, de Graaf SP. The fate of spermatozoa in the female reproductive tract: A comparative review. Theriogenology. 2019 Oct 1;137:104-112.
Wigby S, Suarez SS, Lazzaro BP, Pizzari T, Wolfner MF. Sperm success and immunity. Curr Top Dev Biol. 2019;135:287-313.

Jodar M. Sperm and seminal plasma RNAs: what roles do they play beyond fertilization? Reproduction. 2019 Jun;157(6):R243-R256. doi: 10.1530/REP-18-0627.
Leahy T, Rickard JP, Bernecic NC, Druart X, de Graaf SP. Ram seminal plasma and its functional proteomic assessment. Reproduction. 2019 Oct;158(4):R113-R123
Sun TC, Wang JH, Wang XX, Liu XM, et al. Effects of sperm proteins on fertilization in the female reproductive tract. Front Biosci (Landmark Ed). 2019 Mar 1;24:735-749.
Hernández-Silva G, Chirinos M. Proteins from male and female reproductive tracts involved in sperm function regulation. Zygote. 2019 Feb;27(1):5-16.
Castillo J, Jodar M, Oliva R. The contribution of human sperm proteins to the development and epigenome of the preimplantation embryo. Hum Reprod Update. 2018 Sep 1;24(5):535-555.
Barton BE, Herrera GG, Anamthathmakula P, et al. Roles of steroid hormones in oviductal function. Reproduction. 2020 Mar 1;159(3):R125-R137.
Besenfelder U, Brem G, Havlicek V. Review: Environmental impact on early embryonic development in the bovine species. Animal. 2020 Mar;14(S1):s103-s112.
Saint-Dizier M, Schoen J, Chen S, Banliat C, Mermillod P. Composing the Early Embryonic Microenvironment: Physiology and Regulation of Oviductal Secretions. Int J Mol Sci. 2019 Dec 28;21(1). pii: E223. doi: 10.3390/ijms21010223.
Zou J, Xiang D, Datla R, Wang E (2018) A Protocol for Epigenetic Imprinting Analysis with RNA-Seq Data. Methods Mol Biol. 1751:199-208.
Whittington E, Forsythe D, Borziak K, Karr TL, Walters JR, Dorus S. (2017) Contrasting patterns of evolutionary constraint and novelty revealed by comparative sperm proteomic analysis in Lepidoptera. BMC Genomics. 18(1):931.
Presler M, Van Itallie E, Klein AM, Kunz R, Coughlin ML, Peshkin L, Gygi SP, Wühr M, Kirschner MW. (2017) Proteomics of phosphorylation and protein dynamics during fertilization and meiotic exit in the Xenopus egg. Proc Natl Acad Sci U S A. 12;114(50):E10838-E10847.
Teperek M, Simeone A, Gaggioli V, et al (2016) Sperm is epigenetically programmed to regulate gene transcription in embryos. Genome Res. 26(8):1034-46.
Klosin A, Casas E, Hidalgo-Carcedo C, Vavouri T, Lehner B (2017) Transgenerational transmission of environmental information in C. elegans. Science. 21;356(6335):320-323.
Vicens A, Borziak K, Karr TL, Roldan ERS, Dorus S. (2017) Comparative Sperm Proteomics in Mouse Species with Divergent Mating Systems. Mol Biol Evol. 1;34(6):1403-1416.
Alikhani M, Mirzaei M, Sabbaghian M (2017) Quantitative proteomic analysis of human testis reveals system-wide molecular and cellular pathways associated with non-obstructive azoospermia. J Proteomics 6;162:141-154.
Teperek M, Simeone A, Gaggioli V, et al (2016) Sperm is epigenetically programmed to regulate gene transcription in embryos. Genome Res. 26(8):1034-46.
Mannowetz N, Miller MR, Lishko PV. (2017) Regulation of the sperm calcium channel CatSper by endogenous steroids and plant triterpenoids. Proc Natl Acad Sci U S A. 114(22):5743-5748.
López-Torres AS, González-González ME, Mata-Martínez E, Larrea F, Treviño C, Chirinos M. (2017) Luteinizing hormone modulates intracellular calcium, protein

tyrosine phosphorylation and motility during human sperm capacitation. Biochem Biophys Res Commun. 483(2):834-839.
Lu X, Ding F, Lian Z, Chen L, Cao Z, Guan Y, Chen R, Cai D, Yu Y. (2018) An epididymis-specific secretory protein Clpsl2 critically regulates sperm motility, acrosomal integrity, and male fertility. J Cell Biochem. 2018 Jan 11. doi: 10.1002/jcb.26668. [Epub ahead of print]
Zhang C, Zhou Y, Xie S, Yin Q, Tang C, Ni Z, Fei J, Zhang Y. (2018) CRISPR/Cas9-mediated genome editing reveals the synergistic effects of β-defensin family members on sperm maturation in rat epididymis. FASEB J. 32(3):1354-1363.
Baek S, Lee ST, Hwang JY, Park KH, Yun JI. (2017) Identification of capacitation inducers customized to sperm retrieved from inbred mouse epididymis. Biochem Biophys Res Commun. 24;488(2):273-277.
Sagare-Patil V, Bhilawadikar R, Galvankar M, Zaveri K, Hinduja I, Modi D. (2017) Progesterone requires heat shock protein 90 (HSP90) in human sperm to regulate motility and acrosome reaction. J Assist Reprod Genet. 34(4):495-503.
Griffith OW, Chavan AR, Protopapas S, Maziarz J, Romero R, Wagner GP (2017) Embryo implantation evolved from an ancestral inflammatory attachment reaction. Proc Natl Acad Sci U S A. 8;114(32):E6566-E6575.
Kin K, Maziarz J, Chavan AR, et al (2016) The Transcriptomic Evolution of Mammalian Pregnancy: Gene Expression Innovations in Endometrial Stromal Fibroblasts. Genome Biol Evol. 8(8):2459-73.
Li S, Winuthayanon W. (2017) Oviduct: roles in fertilization and early embryo development. J Endocrinol. 232(1):R1-R26.
Pillai VV, Weber DM, Phinney BS, Selvaraj V. (2017) Profiling of proteins secreted in the bovine oviduct reveals diverse functions of this luminal microenvironment. PLoS One. 12(11):e0188105.
Barrera AD, García EV, Hamdi M, et al (2017) Embryo culture in presence of oviductal fluid induces DNA methylation changes in bovine blastocysts. Reproduction. 154(1):1-12.
Conrad KP. (2016) G-Protein-coupled receptors as potential drug candidates in preeclampsia: targeting the relaxin/insulin-like family peptide receptor 1 for treatment and prevention. Hum Reprod Update. 22(5):647-64.
Whitby S1,2, Salamonsen LA1,3,4, Evans J (2018) The Endometrial Polarity Paradox: Differential Regulation of Polarity Within Secretory-Phase Human Endometrium. Endocrinology. 159(1):506-518.
Ruane PT, Berneau SC, Koeck R (2017) Apposition to endometrial epithelial cells activates mouse blastocysts for implantation. Mol Hum Reprod. 23(9):617-627.
Matsumoto H (2017) Molecular and cellular events during blastocyst implantation in the receptive uterus: clues from mouse models. J Reprod Dev. 63(5):445-454.
Gilchrist GC, Tscherner A, Nalpathamkalam T, et al. (2016) MicroRNA Expression during Bovine Oocyte Maturation and Fertilization. Int J Mol Sci. 18;17(3).
Vasen G, Battistone MA, Croci DO, et al. (2015) The galectin-1-glycan axis controls sperm fertilizing capacity by regulating sperm motility and membrane hyperpolarization. FASEB J. 29(10):4189-200.
Sharma A1, Scott CT2. (2015) The ethics of publishing human germline research. Nat Biotechnol. 33(6):590-2.

Loveland KL, Major AT, Butler R, Young JC, Jans DA, Miyamoto Y. (2015) Putting things in place for fertilization: discovering roles for importin proteins in cell fate and spermatogenesis. Asian J Androl. 17(4):537-44.
Orr TJ, Brennan PL. (2015) Sperm storage: distinguishing selective processes and evaluating criteria. Trends Ecol Evol. 30(5):261-72.
Requena GS, Alonzo SH. (2014) Female sperm use and storage between fertilization events drive sperm competition and male ejaculate allocation. Evolution. 68(12):3433-44.
Sabetian S, Shamsir MS, Abu Naser M. (2014) Functional features and protein network of human sperm-egg interaction. Syst Biol Reprod Med. 60(6):329-37.
Smith ZD, Chan MM, Humm KC, et al. (2014) DNA methylation dynamics of the human preimplantation embryo. Nature. 31;511(7511):611-5.
Gerovska D, Araúzo-Bravo MJ. (2016) Does mouse embryo primordial germ cell activation start before implantation as suggested by single-cell transcriptomics dynamics? Mol Hum Reprod. 22(3):208-25.
Gómez E, Ruíz-Alonso M, Miravet J, Simón C. (2015) Human Endometrial Transcriptomics: Implications for Embryonic Implantation. Cold Spring Harb Perspect Med. 27;5(7):a022996.
Washkowitz AJ, Schall C, Zhang K, Wurst W, Floss T, Mager J, Papaioannou VE. (2015) Mga is essential for the survival of pluripotent cells during peri-implantation development. Development. 142(1):31-40.
Paule S, Nebl T, Webb AI, Vollenhoven B, Rombauts LJ, Nie G. (2015) Proprotein convertase 5/6 cleaves platelet-derived growth factor A in the human endometrium in preparation for embryo implantation. Mol Hum Reprod. 21(3):262-70.
Hapangama DK, Kamal AM, Bulmer JN. (2015) Estrogen receptor β: the guardian of the endometrium. Hum Reprod Update. 21(2):174-93.
Ponsuksili S, Tesfaye D, Schellander K, et al. (2014) Differential expression of miRNAs and their target mRNAs in endometria prior to maternal recognition of pregnancy associates with endometrial receptivity for in vivo- and in vitro-produced bovine embryos. Biol Reprod. 91(6):135.
Xie L, Mouillet JF, Chu T, Parks WT, Sadovsky E, Knöfler M, Sadovsky Y. (2014) C19MC microRNAs regulate the migration of human trophoblasts. Endocrinology. 155(12):4975-85.
Holt WV, Fazeli A. (2016) Sperm Storage in the Female Reproductive Tract. Annu Rev Anim Biosci. 15;4:291-310.
Schjenken JE, Robertson SA. (2015) Seminal Fluid Signalling in the Female Reproductive Tract: Implications for Reproductive Success and Offspring Health. Adv Exp Med Biol. 868:127-58.
Tecle E, Gagneux P. (2015) Sugar-coated sperm: Unraveling the functions of the mammalian sperm glycocalyx. Mol Reprod Dev. 2015 Sep;82(9):635-50.
Holt WV, Fazeli A. (2015) Do sperm possess a molecular passport? Mechanistic insights into sperm selection in the female reproductive tract. Mol Hum Reprod. 21(6):491-501.
Mohanty G, Swain N, Samanta L. (2015) Sperm Proteome: What Is on the Horizon? Reprod Sci. 22(6):638-53.

Jagadeeshan S, Coppard SE, Lessios HA. (2015) Evolution of gamete attraction molecules: evidence for purifying selection in speract and its receptor, in the pantropical sea urchin Diadema. Evol Dev. 17(1):92-108.
Takei GL, Fujinoki M. (2016) Regulation of hamster sperm hyperactivation by extracellular Na+. Reproduction. 2016 Mar 7. pii: REP-15-0367. [Epub ahead of print]
Fujinoki M, Takei GL. (2015) Estrogen suppresses melatonin-enhanced hyperactivation of hamster spermatozoa. J Reprod Dev. 61(4):287-95.
Ernesto JI, Weigel Muñoz M, Battistone MA, et al. (2015) CRISP1 as a novel CatSper regulator that modulates sperm motility and orientation during fertilization. J Cell Biol. 28;210(7):1213-24.
Avella MA, Baibakov B, Dean J. (2014) A single domain of the ZP2 zona pellucida protein mediates gamete recognition in mice and humans. J Cell Biol. 23;205(6):801-9.
Vadnais ML, Gerton GL. (2015) From PAWP to "Pop": opening up new pathways to fatherhood. Asian J Androl. 17(3):443-4.
Singh AP, Rajender S. (2015) CatSper channel, sperm function and male fertility. Reprod Biomed Online. 30(1):28-38.
Denninger P, Bleckmann A, Lausser A, et al. (2014) Male-female communication triggers calcium signatures during fertilization in Arabidopsis. Nat Commun. 22;5:4645.
Robertson SA, Chin PY, Schjenken JE, Thompson JG. (2015) Female tract cytokines and developmental programming in embryos. Adv Exp Med Biol. 843:173-213.
Ghersevich S, Massa E, Zumoffen C. (2015) Oviductal secretion and gamete interaction. Reproduction. 149(1):R1-R14.
Makieva S, Saunders PT, Norman JE. (2014) Androgens in pregnancy: roles in parturition. Hum Reprod Update. 20(4):542-59.
Fisher SJ. (2015) Why is placentation abnormal in preeclampsia? Am J Obstet Gynecol. 213(4 Suppl):S115-22.
Lash GE. (2015) Molecular Cross-Talk at the Feto-Maternal Interface. Cold Spring Harb Perspect Med. 18;5(12).
Spencer TE. (2014) Biological roles of uterine glands in pregnancy. Semin Reprod Med. 32(5):346-57.
Davidson LM, Coward K. (2016) Molecular mechanisms of membrane interaction at implantation. Birth Defects Res C Embryo Today. 108(1):19-32.
Johnson GA, Burghardt RC, Bazer FW. (2014) Osteopontin: a leading candidate adhesion molecule for implantation in pigs and sheep. J Anim Sci Biotechnol. 17;5(1):56.
Carter AM, Enders AC, Pijnenborg R. (2015) The role of invasive trophoblast in implantation and placentation of primates. Philos Trans R Soc Lond B Biol Sci. 370(1663):20140070.
Evans JP, Sherman CD. (2013) Sexual selection and the evolution of egg-sperm interactions in broadcast-spawning invertebrates. Biol Bull. 224(3):166-83.
Burkitt M, et al. (2012) Using computational modeling to investigate sperm navigation and behavior in the female reproductive tract. Theriogenology. 1;77(4):703-16.

Anand-Ivell R, Ivell R. (2011) The special systems biology of the sperm. Biochem J. 15;436(3):e3-5.
Fedorka KM, Winterhalter WE, Ware B. (2011) Perceived sperm competition intensity influences seminal fluid protein production prior to courtship and mating. Evolution. 65(2):584-90.
Foster KR, Pizzari T. (2010) Cooperation: the secret society of sperm. Curr Biol. 13;20(7):R314-6.
Dey SK. (2010) How we are born. J Clin Invest. 2010 Apr;120(4):952-5.
Tavana S, et al. (2012) Effects of Saffron (Crocus sativus L.) Aqueous Extract on In vitro Maturation, Fertilization and Embryo Development of Mouse Oocytes. Cell J. 13(4):259-64.
Peddinti D, Memili E, Burgess SC. (2010) Proteomics-based systems biology modeling of bovine germinal vesicle stage oocyte and cumulus cell interaction. PLoS One. 21;5(6):e11240.
Baker MA, et al. (2012) Proteomic insights into the maturation and capacitation of mammalian spermatozoa. Syst Biol Reprod Med. 58(4):211-7.
Clark GF. (2010) The mammalian zona pellucida: a matrix that mediates both gamete binding and immune recognition? Syst Biol Reprod Med. 56(5):349-64.
Rivera RM. (2010) Epigenetic aspects of fertilization and preimplantation development in mammals: lessons from the mouse. Syst Biol Reprod Med. 56(5):388-404.
Gadella BM. (2012) Dynamic regulation of sperm interactions with the zona pellucida prior to and after fertilisation. Reprod Fertil Dev. 25(1):26-37.
Corry GN, et al. (2009) Epigenetic regulatory mechanisms during preimplantation development. Birth Defects Res C Embryo Today. 87(4):297-313.
Harayama H. (2013) Roles of intracellular cyclic AMP signal transduction in the capacitation and subsequent hyperactivation of mouse and boar spermatozoa. J Reprod Dev. 59(5):421-30.
Oulhen N, Mori M, Dumollard R. (2013) Meeting report - oocyte maturation and fertilization: lessons from canonical and emerging models. J Cell Sci. 1;126(Pt 19):4321-4.
Aitken RJ, Nixon B. (2013) Sperm capacitation: a distant landscape glimpsed but unexplored. Mol Hum Reprod. 19(12):785-93.
Clift D, Schuh M. (2013) Restarting life: fertilization and the transition from meiosis to mitosis. Nat Rev Mol Cell Biol. 14(9):549-62.
Inoue N, et al. (2013) Molecular dissection of IZUMO1, a sperm protein essential for sperm-egg fusion. Development. 140(15):3221-9.
Milardi D, et al. (2013) Proteomics of human seminal plasma: identification of biomarker candidates for fertility and infertility and the evolution of technology. Mol Reprod Dev. 80(5):350-7.
Redgrove KA, et al. (2013) Investigation of the mechanisms by which the molecular chaperone HSPA2 regulates the expression of sperm surface receptors involved in human sperm-oocyte recognition. Mol Hum Reprod. 19(3):120-35.
Ito J, Kashiwazaki N. (2012) Molecular mechanism of fertilization in the pig. Anim Sci J. 83(10):669-82.

Eswari S, Sai Kumar G, Sharma GT. (2013) Expression of mRNA encoding leukaemia inhibitory factor (LIF) and its receptor (LIFRβ) in buffalo preimplantation embryos produced in vitro: markers of successful embryo implantation. Zygote. 21(2):203-13.
Margalit M, et al. (2012) Genetic and physiological study of morphologically abnormal human zona pellucida. Eur J Obstet Gynecol Reprod Biol. 165(1):70-6.
Marcello MR, Singaravelu G, Singson A. (2013) Fertilization. Adv Exp Med Biol. 757:321-50.
Aarabi M, et al. (2012) The testicular and epididymal expression profile of PLCζ in mouse and human does not support its role as a sperm-borne oocyte activating factor. PLoS One. 7(3):e33496.
Nomikos M, Swann K, Lai FA. (2012) Starting a new life: sperm PLC-zeta mobilizes the Ca2+ signal that induces egg activation and embryo development: an essential phospholipase C with implications for male infertility. Bioessays. 34(2):126-34.
Gu TP, et al. (2011) The role of Tet3 DNA dioxygenase in epigenetic reprogramming by oocytes. Nature. 4;477(7366):606-10.
Ito J, Parrington J, Fissore RA. (2011) PLCζ and its role as a trigger of development in vertebrates. Mol Reprod Dev. 78(10-11):846-53.
Zimmerman SW, et al. (2011) Sperm proteasomes degrade sperm receptor on the egg zona pellucida during mammalian fertilization. PLoS One. 23;6(2):e17256.
Clark GF. (2011) Molecular models for mouse sperm-oocyte binding. Glycobiology. 21(1):3-5.
Ikawa M, Inoue N, Benham AM, Okabe M. (2010) Fertilization: a sperm's journey to and interaction with the oocyte. J Clin Invest. 120(4):984-94.
Heytens E, et al. (2009) Reduced amounts and abnormal forms of phospholipase C zeta (PLCzeta) in spermatozoa from infertile men. Hum Reprod. 24(10):2417-28.
Evsikov AV, Marín de Evsikova C. (2009) Gene expression during the oocyte-to-embryo transition in mammals. Mol Reprod Dev. 76(9):805-18.
Brosens JJ, et al. (2014) Uterine selection of human embryos at implantation. Sci Rep. 6;4:3894.
Altmäe S, et al. (2012) Research resource: interactome of human embryo implantation: identification of gene expression pathways, regulation, and integrated regulatory networks. Mol Endocrinol. 26(1):203-17.
Najwa AR, Sengupta J, Ghosh D. (2009) A systems biology approach towards understanding the process of blastocyst implantation. Indian J Physiol Pharmacol. 53(3):197-208.
Corry GN, et al. (2009) Epigenetic regulatory mechanisms during preimplantation development. Birth Defects Res C Embryo Today. 87(4):297-313.
Hu D, Cross JC. (2010) Development and function of trophoblast giant cells in the rodent placenta. Int J Dev Biol. 54(2-3):341-54.
Dominguez F, et al. (2010) Embryologic outcome and secretome profile of implanted blastocysts obtained after coculture in human endometrial epithelial cells versus the sequential system. Fertil Steril. 93(3):774-782.e1.
Makieva S, Saunders PT, Norman JE. (2014) Androgens in pregnancy: roles in parturition. Hum Reprod Update. Mar 18. [Epub ahead of print].
Brosens JJ, et al. (2014) Uterine selection of human embryos at implantation. Sci Rep. 6;4:3894.

Fertilization in the Oviduct

Janice P Evans, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

The process of fertilization unites the two gametes to create and initiate development of the embryo. However, even before the sperm and egg meet and come together to become one combined cell, there are a noteworthy number of preceding, preparatory events that must occur for each of the gametes. Just has a student has to take precalculus before calculus, or take basic chemistry before organic chemistry, the gametes in both the male and female have to undergo prerequisite steps in order for fertilization itself to be successful. This article will cover these necessary prerequisite steps for the gametes, and then will also address the merger of the gametes and the early steps that initiate embryonic development. This also will highlight how fertilization in vivo (i.e., in the oviduct) differs from fertilization in vitro. In addition to this overview, there is more detailed information on these events in other chapters of this volume.

The Oocyte's Preparations for Fertilization

Each oocyte develops in its own individual compartment in the ovary, known as the ovarian follicle (or simply "follicle" for short). Oocytes progress through meiosis in a very staggered fashion. Mammalian female gametes initiate meiosis in the fetal ovary, doing DNA replication and progressing through the stages of prophase I (leptotene, zygotene, and pachytene), ultimately to arrive and arrest at what will be an extended version of diplotene phase; this is called the dictyate stage, also known as the resting stage of meiosis. With this as the starting point, there are two things that must change with the oocyte in order for fertilization to occur: (1) the oocyte must leave the ovarian follicle and enter the oviduct (also known as the fallopian tube; specifically the ampulla region of the oviduct, the site where fertilization normally occurs); and (2) the oocyte must progress from this prophase I arrest through meiosis I to a second arrest at metaphase of meiosis II. (One exception to this are dogs and foxes, with oocytes that arrest in meiosis and get fertilized at metaphase I of meiosis.)

These two crucial changes of the oocyte are coordinated by actions downstream from luteinizing hormone (LH), the gonadotropin that triggers ovulation. LH binding to the LH receptor on granulosa cells surrounding the outer wall of the pre-ovulatory follicle (known as mural granulosa cells) induces production of a variety of molecules within the follicle, with one of the most significant being the small signaling lipid Prostaglandin E2 (Duffy, 2015; Kim and Duffy, 2016). Through paracrine effects on somatic cells within the follicle, PGE2 facilitates follicle rupture, allowing the oocyte to leave the ovarian follicle and transverse to the nearby ampulla of the oviduct. The oocyte is released from the ovarian follicle surrounded by its associated cumulus cells, as a *cumulus–oocyte complex* (COC).

The actions of LH also induce a cessation of the signaling that maintains prophase I arrest. During early stages of oogenesis and folliculogenesis, oocytes initially are not competent to exit from prophase I arrest. Oocytes develop this meiotic competence during later stages of oocyte growth and follicle development, and once oocytes achieve meiotic competence, signaling within the ovarian follicle keeps the oocytes arrested at prophase I (Jaffe and Egbert, 2017). This arrest of meiotically competent oocytes in preovulatory follicles is mediated by a protein called natriuretic peptide precursor C (NPPC, also known as C-type natriuretic peptide). NPPC is produced by the mural granulosa cells, then acts in a paracrine fashion, binding to its receptor on cumulus cells (the NPPC receptor is known as natriuretic peptide receptor 2, or NPR2). NPR2 has enzymatic activity as a guanylate cyclase that produces the secondary messenger molecule cyclic GMP (cGMP). cGMP is transferred from the cumulus cells to the oocyte through gap junctions, and cGMP in the oocyte inhibits the activity of an enzyme called phosphodiesterase 3A (PDE3A). PDE3A is an enzyme that degrades the secondary messenger molecule cyclic AMP (cAMP), and inhibiting this enzyme and keeping cAMP levels in the oocyte high is crucial for maintain prophase I arrest in meiotically competent oocytes. cAMP in the oocyte acts through the cAMP kinase protein kinase A (PKA), which phosphorylates several cell cycle regulatory proteins that mediate maintenance of the oocyte's prophase I arrest (Jaffe and Egbert, 2017).

When it is time for ovulation and thus for the oocyte to exit from this prophase I arrest, the actions of LH reverse this entire process. This is achieved by several events within the follicle. Two components of this are LH causing both a decrease in NPR2 guanylate cyclase activity in cumulus cells and an increase in degradation of cGMP. These two actions decrease cGMP concentrations in cumulus cells, and in turn reducing the amount of cGMP in the oocyte (Jaffe and Egbert, 2017). In addition, LH binding to the LH receptor on mural granulosa cells leads to a new set of paracrine signals within the follicle, through the release of epiregulin and amphiregulin from the granulosa cells. These two proteins are ligands for epidermal growth factor receptors (EGFRs) present on the cumulus cells surrounding the oocyte. The binding of epiregulin and/or amphiregulin to cumulus cell EGFRs also appears to contribute to the decrease in cGMP (Jaffe and Egbert, 2017). An additional observed effect of LH in the preovulatory follicle is closure of gap junctions connecting closure of gap junctions connecting the cumulus cells to the oocytes, in turn terminating the transfer of cGMP to the oocytes (Jaffe and Egbert, 2017). The end result of all this is decreasing the amount of cGMP in the oocyte, which allows PDE3A to become active and to degrade cAMP. The decline in cAMP activity in the oocyte allows the oocyte to resume meiosis. The oocyte progresses from prophase I into meiosis I, and through cytokinesis (polar body emission), separating the homologous chromosomes. The sister chromatids remain together, and align as the meiotic spindle of metaphase II forms. The

ovulated oocyte in the oviduct is arrested at metaphase II, awaiting the signal from the sperm to complete meiosis (to be addressed below). The terms "metaphase II egg," "MII egg," or simply "egg" are sometimes used to describe the oocyte at this stage of meiosis.

The Sperm's Preparations for Fertilization

One obvious change that has to happen with the sperm is in location—the sperm has to be deposited in the female reproductive tract. But even before this, there are other crucial changes that the sperm has to undergo. Sperm complete meiosis during spermatogenesis in the seminiferous tubule of the testis (thus differing from how the female gamete undergoes meiosis). However, even though sperm isolated from the testis are haploid, these haploid sperm in the testis are unable to fertilize an egg. (The exception to this is when a sperm is injected into the egg cytoplasm through an assisted reproductive technology [ART] method known as intracytoplasmic sperm injection [ICSI].)

Two key steps of the sperm's preparations for fertilization occur while the sperm is still in the male, after the sperm has left the testes. The first of these is transit through the epididymis, with the sperm undergoing a process known as *epididymal maturation*. A crucial change that occurs during epididymal maturation is sperm become competent for progressive motility. Changes in sperm during epididymal maturation are thought to be mediated by several events, including sperm interactions with the epididymal epithelium, changes in the sperm surface proteome through acquisition of secreted proteins from the epididymal epithelium, and sperm acquisition of small vesicles released from the epididymal cells (exosomes or epididymosomes) (Gervasi and Visconti, 2017).

The second prerequisite step for the sperm occurring in the male is *mixture of the sperm with the semen*. Seminal plasma components are secreted by the epididymis and accessory glands of the male reproductive tract, the seminar vesicle, prostate, and bulbourethral gland (McGraw et al., 2015). In humans, the components of the semen are important for sperm to survive in the vagina and/or uterus (McGraw et al., 2015). The human vaginal environment is acidic, maintained in large part by the production of lactic acid by bacteria called lactobacilli. The acidic environment is beneficial for preventing certain vaginal infections, as the low pH will kill many types of microbes. However, the acidic environment will also kill sperm. Therefore, the buffering components of seminal fluid are essential to allow the sperm to survive deposition in the vagina. In some animal species that are induced ovulators (e.g., rabbits, camels), seminal plasma contains factors that induce ovulation (Adams and Ratto, 2013). There also are intriguing data that suggest that seminal plasma can affect the health of resulting offspring, based on experimental studies in mice comparing reproductive outcomes from males with and without seminal vesicles (Bromfield et al., 2014; McGraw et al., 2015).

After ejaculation, sperm then make their way through the female tract. The location of sperm deposition in the female tract varies between species (vagina, cervix, or uterus) (Gervasi and Visconti, 2016; McGraw et al., 2015). In humans, sperm travel from the vagina, through the cervix, into the uterus, and to region of the oviduct adjacent to the uterus, known as the utero-tubal junction (UTJ). It should be noted that the human cervix is hormonally primed during follicular phase of the menstrual cycle, such that the cervical mucus around the time ovulation is thin and watery, thus facilitating sperm passage through the cervix into the uterus. Data from a variety of studies, most notably of mouse knockout models, suggest that some process of sperm selection occurs in the female tract; the sperm from several specific knockouts fail to reach the UTJ, indicating that several sperm proteins have direct or indirect roles in successful transit to this part of the female tract (Gervasi and Visconti, 2016; Suarez, 2016).

It is in the female reproductive tract that sperm undergo their next important prerequisite step, *capacitation*, which is broadly defined as the process by which sperm acquire the capacity to fertilize. Capacitation in vivo is thought to be an extended, continuous process, beginning with ejaculation and continuing as sperm reside in the female tract. In the human, the peri-ovulatory cervical mucus may be part of the stimulus for the initiation of capacitation, with the uterine and oviductal environments continuing to support capacitation (De Jonge, 2017). Since the phenomenon of capacitation was first discovered, it has since been determined that capacitation can occur in vitro in certain chemically defined medium (Gervasi and Visconti, 2016). These discoveries and technological developments were part of what paved the way to making in vitro fertilization possible. Capacitation in vivo is mediated by a sequential series of cellular signaling events occurring in sperm as they travel up the female tract, central among them being signaling mediated by the second messenger molecule cAMP (Gervasi and Visconti, 2016). This signaling produces several biochemical changes in the sperm, which combine to culminate in capacitated sperm.

What is different about sperm that have undergone capacitation? The classic definition of capacitation means that the sperm now has the capacity to fertilize an egg, but there are specific changes observed as well, particularly with the later stages of capacitation. One of these is *hyperactivation of motility*. This is a distinct pattern of motility from the progressive motility acquired during epididymal sperm maturation (noted above), with hyperactivated motility characterized by high amplitude, asymmetric beating of the sperm tail (Suarez, 2008a). A key molecular player in hyperactivated motility is the multimeric ion channel known as Catsper, so named for its function as a cation/calcium channel on sperm. Catsper-mediated influx of calcium into the sperm tail drives hyperactivated motility. In human sperm, the activity of Catsper is stimulated by the steroid hormone progesterone, which is present in follicular fluid that is released with the cumulus–oocyte complex upon ovulation (Miller et al., 2015).

Another event associated with the later stages of capacitation is the exocytosis of a large vesicle on the head of the sperm, the *acrosome*. (Note: *Acrosome exocytosis* is also referred to as the *acrosome reaction*.) A long-time model for acrosome exocytosis is that the sperm binding the egg's coat, the zona pellucida (ZP), is the stimulus for acrosome exocytosis. Although there still is strong evidence for sperm interaction with the ZP, including in in vivo contexts (Avella et al., 2014, 2016), there is ongoing evaluation of the biology of acrosome exocytosis, including consideration of acrosome exocytosis in vivo being an event associated with

capacitation of sperm in the female tract (Gervasi and Visconti, 2016; Hirohashi, 2016). This is based on data from a variety of experimental studies (Inoue et al., 2011; Jin et al., 2011; La Spina et al., 2016), with the cautionary note that much of this is based on studies of mouse fertilization, and several aspects of sperm biology differ between species (De Jonge, 2017; Kaupp and Strunker, 2017). Setting aside the question of the physiological location of acrosome exocytosis in vivo, it should be emphasized that acrosome exocytosis is an important prerequisite step for fertilization. Acrosome exocytosis induces changes in the sperm surface, including remodeling and exposure of new surfaces of the sperm that render the sperm capable of binding and fusing with the oocyte plasma membrane (Cuasnicu et al., 2016; Hirohashi, 2016).

A related aspect of sperm residence in the female tract is the formation of *oviductal sperm reservoirs*, specifically in a region of the oviduct called the isthmus (Suarez, 2016). In most species, the establishment of this oviductal sperm reservoir is mediated by sperm binding to the epithelial cells that line the oviduct. The formation of oviductal sperm reservoirs is facilitated by a preceding step with the sperm, the mixture of sperm with semen. Proteins in the seminal plasma help to mediate sperm interactions with the oviductal epithelium (McGraw et al., 2015).

The formation of these oviductal sperm reservoirs is thought to contribute to reproductive success in several ways, including maintaining sperm viability and fertility, regulating capacitation and hyperactivation of motility, and helping to prevent polyspermic fertilization (fertilization of the egg by more than one sperm). Release of sperm from interactions with oviductal epithelial cells appears to be associated with later stages of capacitation and tied with signals with ovulation, although this is not fully understood (Gervasi and Visconti, 2016). Acquisition of hyperactivated motility appears to facilitate detachment of sperm from the oviductal epithelium (Suarez, 2016).

One practical implication of this in human reproductive biology is that these oviductal sperm reservoirs contribute to sperm being able to live in the female tract for several days (and in some species, sperm can live in the female tract for weeks and even months) (Holt, 2011; Suarez, 2008b). Sperm storage thus affects the fertile window that needs to be considered for fertility awareness-based methods of contraception. For example, if a woman has unprotected intercourse on a Monday and then ovulates 5 days later on Friday, this ovulated egg could potentially be fertilized by sperm residing in these reservoirs in her reproductive tract. Fertility awareness-based methods of contraception thus require that a woman abstain from intercourse or use a contraceptive (methods such as condoms, diaphragm, or spermicidal foams or creams) during the time frame in which sperm could survive in the female tract long enough to fertilize an egg that could be ovulated days later.

The Gametes in the Ampulla of the Oviduct

Following the release of sperm from the oviductal sperm reservoirs in the isthmus of the oviduct, sperm then make their way to the ampulla of the oviduct, the site of fertilization. Sperm first make contact with the oocyte's outermost coat, the cumulus layer (also called the cumulus oophorus). This layer contains cumulus cells, the somatic cells that surrounded the oocyte in the ovarian follicle, embedded in an extracellular matrix of hyaluronic acid. Hyperactivated motility facilitates penetration of this extracellular matrix, and there also is a possible role of a sperm-associated hyaluronidase activity, although this has not been conclusively demonstrated (Chang and Suarez, 2010; Hirohashi, 2016; Suarez, 2008a). The next extracellular coat that the sperm makes contact with is the zona pellucida (ZP). As noted above, there are ongoing questions about a sperm has to have an intact acrosome to interact with the ZP and whether the ZP serves as an in vivo agonist to trigger acrosome exocytosis (Buffone et al., 2014), but there are data that provide evidence of sperm–ZP binding (e.g., Avella et al., 2014, 2016). Hyperactivated motility plays a crucial role in sperm passage through the ZP to get the sperm to the space between the ZP and the oocyte plasma membrane, known as the perivitelline space.

Once in the perivitelline space, the sperm binds and fuses with the oocyte plasma membrane with the sperm protein known as IZUMO1 and the oocyte protein called Juno being essential for this process of the individual gametes merging to become the one-cell embryo (Yeste et al., 2017). Obviously one important part of the sperm that is delivered to the oocyte is the paternal DNA. In some species, sperm-provided centrioles may help mediate organization of the mitotic spindle for the first embryonic division (Clift and Schuh, 2013). However, even before embryonic mitosis is going to occur, the oocyte must exit from its arrest in metaphase of meiosis II, where it has been arrested since ovulation (see above). In addition, the oocyte has processes to prevent fertilization by additional sperm, known as blocks to polyspermy. These events are collectively known as *oocyte activation* (or *egg activation*), and are triggered by fertilization by the sperm.

Sperm–oocyte fusion results in cytoplasmic continuity between these two cells, allowing delivery of sperm components into the egg cytoplasm. In one of the earliest steps of this process, the sperm brings the factor that triggers the oocyte's completion of meiosis and initiation of embryonic development—the sperm-specific phospholipase C, PLCζ, which plays a key role in (Clift and Schuh, 2013; Yeste et al., 2017). PLCζ, like other phospholipase Cs, hydrolyzes the lipid phosphatidylinositol 4,5-bisphosphate (PIP_2) to produce two cleavage products, inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 binds to the IP_3 receptor on the endoplasmic reticulum, which is an intracellular store for calcium ions (Ca^{2+}). The IP_3 receptor, with IP_3 bound to it, functions as a Ca^{2+} channel, allowing Ca^{2+} to be released from the endoplasmic reticulum to the oocyte cytoplasm. Once in the oocyte cytoplasm, calcium ions serve as the major driver of the oocyte-to-embryo transition, activating calcium/calmodulin-dependent kinase IIγ (CaMKIIγ) (Clift and Schuh, 2013). This kinase phosphorylates downstream substrates that lead to exit from metaphase II arrest, completion of meiosis, and progression to embryonic mitosis.

In addition to cell cycle resumption, another event of oocyte activation is the establishment of blocks to polyspermy. As noted above, IP_3 receptor-mediated release of calcium ions from the endoplasmic reticulum results in increased cytosolic calcium concentration. This calcium triggers exocytosis of vesicles known as cortical granules. A protease called ovastacin is released from the cortical granules, and this protease cleaves the ZP component ZP2, resulting in a form of the ZP that does not support sperm binding (Burkart et al., 2012). Calcium also appears to mediate the conversion of the oocyte plasma membrane to a form that is less supportive of sperm interaction (Gardner et al., 2007).

Conclusion

The events summarized here can be considered the culminating events of sexual reproduction, the process by which the gametes from two genetically distinct individuals come together to create new, genetically unique offspring. Gaining improved understanding of these central events of reproduction will advance our understanding of how reproduction can go awry, and result in subfertility or infertility. For example, a subset of men seen in infertility clinics have sperm with normal motility and morphology, and that can fuse with eggs, but the eggs fail to undergo egg activation. Research studies of these patients have revealed that sperm from some of these men lack functional PLCζ (Kashir et al., 2011; Yoon et al., 2008). On the flip side, knowledge of how reproduction works will provide insights into ways to impair reproductive processes, and thus open the door to future methods of contraception. Several of the biological events noted in this article have been proposed to be potential targets for contraception. These include perturbation of ovulation by interfering with production of Prostaglandin E2 (Duffy, 2015), impairing the sperm's acquisition of hyperactivated motility with steroid-like molecules that act in an antagonistic fashion to progesterone (Mannowetz et al., 2017), and placing beads coated with ZP-like peptides in the uterus can serve as a "decoy" for sperm, causing sperm to fail to travel from the uterus to the oviduct (Avella et al., 2016). Research in this and other areas of reproductive biology is poised to produce discoveries that will lead significant advances for human reproductive health.

References

Adams, G. P., & Ratto, M. H. (2013). Ovulation-inducing factor in seminal plasma: A review. *Animal Reproduction Science, 136*, 148–156.

Avella, M. A., Baibakov, B., & Dean, J. (2014). A single domain of the ZP2 zona pellucida protein mediates gamete recognition in mice and humans. *The Journal of Cell Biology, 205*, 801–809.

Avella, M. A., Baibakov, B. A., Jimenez-Movilla, M., Sadusky, A. B., & Dean, J. (2016). ZP2 peptide beads select human sperm in vitro, decoy mouse sperm in vivo, and provide reversible contraception. *Science Translational Medicine, 8*, 336ra360.

Bromfield, J. J., Schjenken, J. E., Chin, P. Y., Care, A. S., Jasper, M. J., & Robertson, S. A. (2014). Maternal tract factors contribute to paternal seminal fluid impact on metabolic phenotype in offspring. *Proceedings of the National Academy of Sciences of the United States of America*.

Buffone, M. G., Hirohashi, N., & Gerton, G. L. (2014). Unresolved questions concerning mammalian sperm acrosomal exocytosis. *Biology of Reproduction, 90*, 112.

Burkart, A. D., Xiong, B., Baibakov, B., Jimenez-Movilla, M., & Dean, J. (2012). Ovastacin, a cortical granule protease, cleaves ZP2 in the zona pellucida to prevent polyspermy. *The Journal of Cell Biology, 197*, 37–44.

Chang, H., & Suarez, S. S. (2010). Rethinking the relationship between hyperactivation and chemotaxis in mammalian sperm. *Biology of Reproduction, 83*, 507–513.

Clift, D., & Schuh, M. (2013). Restarting life: Fertilization and the transition from meiosis to mitosis. *Nature Reviews. Molecular Cell Biology, 14*, 549–562.

Cuasnicu, P. S., Da Ros, V. G., Weigel Munoz, M., & Cohen, D. J. (2016). Acrosome reaction as a preparation for gamete fusion. *Advances in Anatomy, Embryology, and Cell Biology, 220*, 159–172.

De Jonge, C. (2017). Biological basis for human capacitation-revisited. *Human Reproduction Update, 23*, 289–299.

Duffy, D. M. (2015). Novel contraceptive targets to inhibit ovulation: The prostaglandin E2 pathway. *Human Reproduction Update, 21*, 652–670.

Gardner, A. J., Williams, C. J., & Evans, J. P. (2007). Establishment of the mammalian membrane block to polyspermy: Evidence for calcium-dependent and -independent regulation. *Reproduction, 133*, 383–393.

Gervasi, M. G., & Visconti, P. E. (2016). Chang's meaning of capacitation: A molecular perspective. *Molecular Reproduction and Development, 83*, 860–874.

Gervasi, M. G., & Visconti, P. E. (2017). Molecular changes and signaling events occurring in spermatozoa during epididymal maturation. *Andrology, 5*, 204–218.

Hirohashi, N. (2016). Site of mammalian sperm acrosome reaction. *Advances in Anatomy, Embryology, and Cell Biology, 220*, 145–158.

Holt, W. V. (2011). Mechanisms of sperm storage in the female reproductive tract: An interspecies comparison. *Reproduction in Domestic Animals = Zuchthygiene., 46*(Suppl 2), 68–74.

Inoue, N., Satouh, Y., Ikawa, M., Okabe, M., & Yanagimachi, R. (2011). Acrosome-reacted mouse sperm recovered from the perivitelline space can fertilize other eggs. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 20008–20011.

Jaffe, L. A., & Egbert, J. R. (2017). Regulation of mammalian oocyte meiosis by intercellular communication within the ovarian follicle. *Annual Review of Physiology, 79*, 237–260.

Jin, M., Fujiwara, E., Kakiuchi, Y., Okabe, M., Satouh, Y., Baba, S. A., Chiba, K., & Hirohashi, N. (2011). Most fertilizing mouse spermatozoa begin their acrosome reaction before contact with the zona pellucida during in vitro fertilization. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 4892–4896.

Kashir, J., Konstantinidis, M., Jones, C., Lemmon, B., Chang Lee, H., Hamer, R., Heindryckx, B., Deane, C. M., DeSutter, P., Fissore, R. A., Parrington, J., Wells, D., & Coward, K. (2011). A maternally inherited autosomal point mutation in human phospholipase C zeta (PLCζ) leads to male infertility. *Human Reproduction, 27*, 222–231.

Kaupp, U. B., & Strunker, T. (2017). Signaling in sperm: More different than similar. *Trends in Cell Biology, 27*, 101–109.

Kim, S. O., & Duffy, D. M. (2016). Mapping PTGERs to the ovulatory follicle: Regional responses to the ovulatory PGE2 signal. *Biology of Reproduction, 95*, 33.

La Spina, F. A., Puga Molina, L. C., Romarowski, A., Vitale, A. M., Falzone, T. L., Krapf, D., Hirohashi, N., & Buffone, M. G. (2016). Mouse sperm begin to undergo acrosomal exocytosis in the upper isthmus of the oviduct. *Developmental Biology, 411*, 172–182.

Mannowetz, N., Miller, M. R., & Lishko, P. V. (2017). Regulation of the sperm calcium channel CatSper by endogenous steroids and plant triterpenoids. *Proceedings of the National Academy of Sciences of the United States of America, 114*, 5743–5748.

McGraw, L. A., Suarez, S. S., & Wolfner, M. F. (2015). On a matter of seminal importance. *BioEssays, 37*, 142–147.

Miller, M. R., Mansell, S. A., Meyers, S. A., & Lishko, P. V. (2015). Flagellar ion channels of sperm: Similarities and differences between species. *Cell Calcium, 58*, 105–113.

Suarez, S. S. (2008a). Control of hyperactivation in sperm. *Human Reproduction Update, 14*, 647–657.

Suarez, S. S. (2008b). Regulation of sperm storage and movement in the mammalian oviduct. *The International Journal of Developmental Biology, 52*, 455–462.

Suarez, S. S. (2016). Mammalian sperm interactions with the female reproductive tract. *Cell and Tissue Research, 363*, 185–194.

Yeste, M., Jones, C., Amdani, S. N., & Coward, K. (2017). Oocyte activation and fertilisation: Crucial contributors from the sperm and oocyte. *Results and Problems in Cell Differentiation, 59*, 213–239.

Yoon, S. Y., Jellerette, T., Salicioni, A. M., Lee, H. C., Yoo, M. S., Coward, K., Parrington, J., Grow, D., Cibelli, J. B., Visconti, P. E., Mager, J., & Fissore, R. A. (2008). Human sperm devoid of PLCζ1 fail to induce Ca^{2+} release and are unable to initiate the first step of embryo development. *The Journal of Clinical Investigation, 118*, 3671–3681.

FERTILIZATION

Fertilization, Comparative

Ryusaku Deguchi, Miyagi University of Education, Miyagi, Japan
Noritaka Hirohashi, Shimane University, Shimane, Japan

Introduction

In multicellular metazoan animals, two different types of gametes, eggs and spermatozoa, are unified by fertilization, and as the result fertilized eggs undergo mitotic cell divisions to develop into adult organisms. The processes of fertilization comprise multiple steps with highly sophisticated physical and chemical reactions within each gamete as well as between both gametes, ensuring normal fertilization. In recent years, research in this area has been carried out mainly using mammalian species including the laboratory model animals (e.g., mice and rats), domestic animals (e.g., pigs and cows), and humans. Thanks to that, a comprehensive view of fertilization has begun describing in terms of the cellular and molecular mechanisms. However, it is still worth studying with a wide range of animals to find the general principle as well as some features unique to particular groups or species. In this article, comparative aspects of the fertilization mechanisms in wide range of animal phyla of invertebrates and vertebrates, which could have evolved from a common ancestor (Fig. 1), are described.

The Site of Fertilization

Fertilization occurs inside or outside the females. The site of fertilization is fundamental for determining the animal reproduction modes, oviparity (females lay unfertilized or developing eggs) and viviparity (females retain developing eggs inside their bodies and give birth to their offspring). Thus, all viviparous animals are internal fertilizers, whereas oviparous animals are either internal or external ones. Specifically, a mode in which females lay unfertilized eggs is called ovuliparity.

There are a number of thermic issues regarding the site of fertilization. In general, the site of fertilization does not coincide with the site of sperm deposition (insemination, ejaculation or spawning), therefore spermatozoa must travel in a certain distance to meet eggs. In internal fertilizers, especially in mammals, only a handful number of spermatozoa arrive in the upper (ampullae) part of the oviduct despite hundreds of those can reach the lower (isthmus) part of the oviduct. Several guidance mechanisms, such as thermotaxis, chemotaxis and rheotaxis, have proposed to explain how fertilizing spermatozoa are navigated to the site of fertilization. In the thermotaxis model, a temperature difference between the proximal ampulla and the distal isthmus regulates swimming direction of the ejaculated spermatozoa. Such a temperature difference was actually recorded 0.69°C in pigs and 0.8°C in rabbits. In the sperm chemotaxis model, spermatozoa can sense a gradient of progesterone derived from the cumulus cells surrounding the oocyte and swim toward the source of progesterone. In the rheotaxis model, spermatozoa swim against the fluid flow that is generated from oviduct to uterus after coitus, facilitating sperm guidance over long distances in the female reproductive tract (Miki and Clapham, 2013).

In external fertilizers, gametes are released thereafter diluted into aquatic environment so that timely (synchronous) spawning that increases frequency of sperm-egg encounter could have been developed. In addition, spermatozoa must find conspecific eggs to fertilize them. Hence, species-specificity in sperm-egg interactions, occurring at the level of sperm attraction to eggs, sperm acrosome reaction and sperm binding to the egg surface, are the central issues of research interests for last several decades. Occasionally, timing of sperm deposition (insemination) does not coincide with that of egg deposition (ovulation), therefore spermatozoa must await fertilization in the female reproductive tract, so that research addresses mechanisms of extended sperm survival at molecular, cellular and organismal levels. In many animals, females are promiscuous (polyandry; mating with multiple males), by which post-copulatory sexual selection, i.e., sperm competition and cryptic female choice, can favor fertilizations toward genetically more compatible males. This addresses evolutionary consequences of female reproductive systems and corresponding adaptive sperm traits (Birkhead and Pizzari, 2002).

Most, if not all, species thus far examined that employ external fertilization are cnidarians, echinoderms, ascidians, and teleost fish, whereas most species employing internal fertilization are insects, reptiles, birds, and mammals (both marsupials and eutherians). Mollusks, crustaceans, and amphibians are the groups that employ either internal or external depending on species. In teleost fish, synchronous spawning of gametes from both sexes into the same water column is a common strategy. Both gametes lose their fertilization competence shortly after the spawning, hence fertilization must be completed within a short period of time. Exceptionally, herring gametes, once released into ocean, are still capable of sustaining their fertilizing competence for days and spermatozoa remain quiescence until reached at the egg micropyle region where a sperm motility initiation factor activates spermatozoa and guides the sperm cells into the micropylar canal (Yanagimachi *et al.*, 2017). Sea urchins and starfish employ broadcast spawning where some millions to billions of gametes are released from single adults into sea. Spermatozoa encounter eggs coincidentally

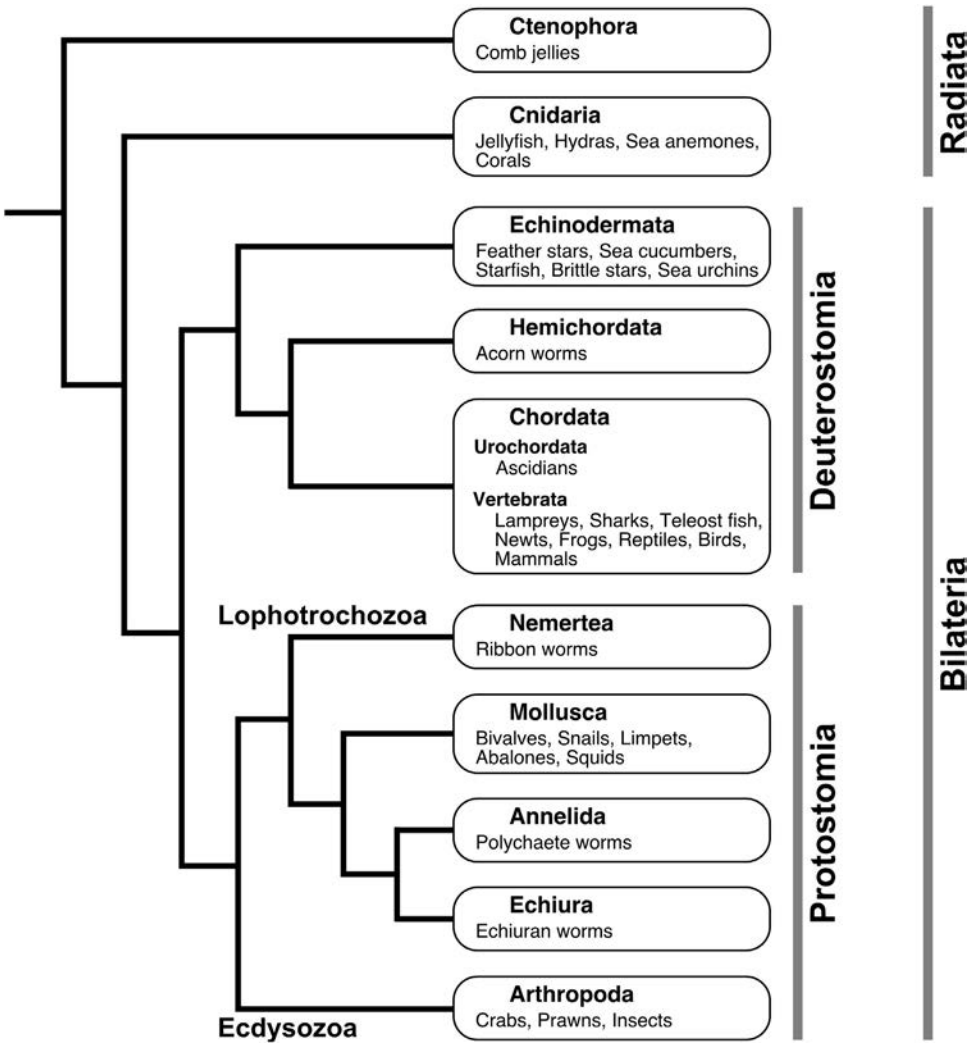


Fig. 1 Example of phylogenetic tree of the multicellular animals described in the text.

or with a chemical cue that guides spermatozoa to a conspecific egg, which is known for sperm chemotaxis. In some corals, spermatozoa and eggs are produced in the same individuals (hermaphrodite) and released together as bundles that drift to the ocean surface where fertilization takes place. Although broadcast spawners release massive amount gametes into sea, synchrony of gamete spawning from both sexes, presumably to gain fertilization opportunities, is a common feature. By contrast, there are some situations where gametes are released asynchronously. For example, in the mediterranean gobies, males deposit spermatozoa in the form of sperm trails laid on the nest surface before females start laying their eggs. To compensate the time-gap between sperm release by males and egg release by females, the sperm longevity (i.e., fertilizability) is guaranteed by either rendering their motility inactive or keeping them active for the extended duration. Several species in jellyfish, ascidians, and teleost fish are taking the former strategy; ejaculated spermatozoa remain quiescent until an egg factor activates sperm motility. Alternatively, spermatozoa are stored quiescently in the female storage organs, such as insect spermatheca, cephalopod or annelid seminal receptacle, bird sperm storage tubules (SSTs), and mammalian sperm reservoir. The average storing period of spermatozoa are varied from one group to another, and even from one species to another; hours to days in mammals, weeks in birds, months in reptiles and cephalopods, years to decades in bees and ants (Birkhead and Møller, 2008). Spermatozoa stored in the female storage organ are thereafter transferred to the site of fertilization. In mammals, fertilization occurs at the outermost portion of the fallopian tube, called the ampulla where ovulated oocytes are picked up by the infundibulum of the oviduct. In birds, spermatozoa in the SSTs at the utero-vaginal junction are transported to the infundibulum through the oviduct. In insects, spermatozoa are stored in spermatheca and fertilization occurs predominantly in the median oviduct. In the squid species, males implant sperm-containing capsules (spermatophores) to the external body surfaces or internal mantle cavities of the females. In some cases, the spermatophores themselves serve as sperm reservoirs, whereas in other cases, spermatozoa released from the spermatophores are stored in the female's seminal receptacles. Although fertilization may take place in the close vicinity of the implanted spermatophores or the seminal receptacles, direct observations are lacking.

Sperm Acrosome Reaction

Mature spermatozoa carry the acrosome, a Golgi-originated single large vesicle within the anterior potion of the sperm head. The acrosomal vesicle is discharged in response to a certain stimulus to release intra-vesicular contents as well as to expose inner surface of the acrosomal vesicle (inside-out) during fertilization. The anterior tip of sperm head becomes elongated in some marine invertebrates, or sharpened (perforatorium) in mammals following breakdown of the acrosome. Such a series of morphological changes occurring in sperm head is called "the acrosome reaction". The term "the acrosomal exocytosis" has been often used instead, particularly in mammals. The sperm acrosome reaction has been so far observed in wide range of animal groups such as mollusks (bivalves and sea snails), annelids (polychaete worms), arthropods (shrimps, crabs, and horseshoe crabs), echinoderms (feather stars, starfish, sea cucumbers, brittle stars, and sea urchins), cephalochordates (acorn worms), cyclostomes (hagfish and lampreys), and many other vertebrates including mammals. However, spermatozoa of some groups such as teleost fish lack the acrosome. It is postulated that as egg micropyle has evolved, the acrosome reaction has lost its significance. There are several distinct roles of the acrosome and acrosome reaction for fertilization. In marine invertebrates, the acrosome reaction occurs predominantly during sperm-egg interactions and acrosome-reacted spermatozoa can dissolve the layer covering the egg (egg coat) so that the acrosome reaction is believed to be prerequisite for penetrating the egg coat. The acrosome contains enzymatic activities such as proteases and glycosidases by which spermatozoa may be able to lyse the extracellular matrix of the egg. In sea snails and abalones, however, mode of action of sperm's lytic activity is non-enzymatic. In sea urchins, the most abundant component of the acrosome is a ~30-kDa protein called bindin that plays the roles in species-specific sperm adhesion to egg surface and sperm-egg fusion. Upon the acrosome reaction, bindin becomes externalized and deposited on the surface of a finger-like protrusion (the acrosomal process) of the sperm head, enabling spermatozoa to attach on the egg vitelline layer (Vacquier, 2012). In mammals, the acrosome reaction is also required for tight adhesion of spermatozoa to the egg coat called zona pellucida and consequently penetration of bound spermatozoa through the zona matrix. Thus, the sperm acrosome reaction must occur at some points before penetrating the egg coat, however the exact location(s) remains unknown. The long-standing hypothesis proposed that acrosome-intact spermatozoa adhere the zona surface thereafter trigger the acrosome reaction. However, acrosome-reacted spermatozoa can also bind the zona and fertilize the egg (La Spina *et al.*, 2016).

In sea urchins, unfertilized eggs are surrounded by a gelatinous transparent layer called jelly coat where spermatozoa undergo the acrosome reaction. Jelly coat contains a sulfated fucose polymer (fucan) that induces the acrosome reaction. In all sea urchin species so far investigated except for one case (a sulfated galactan), egg jelly contains at least one form of sulfated fucans. A structural feature conserved among different sulfated fucans is that they are consisted of tandem repeat of a sulfated oligosaccharide unit (Vilela-Silva *et al.*, 2002). Differences are observed in the glycosidic linkage and positions of sulfation. Such the structural differences can explain the species-specificity in induction of the acrosome reaction. Spermatozoa recognize a conspecific sulfated fucan through a receptor for egg jelly (REJ) located on the flagellar membrane (Gunaratne *et al.*, 2007), which evokes concomitant changes in ionic permeability; the influx of Ca^{2+} and Na^{+} and the efflux of H^{+} and K^{+}. In particular, Ca^{2+} plays an essential role in fusion between the sperm plasma membrane and the outer acrosomal membrane (Gonzalez-Martinez *et al.*, 2001). This fusion event is highly regulated and conserved in the secretory pathway of other cell types (Belmonte *et al.*, 2016). During the acrosomal exocytosis, the membrane dynamics can be divided into several distinctive stages, i.e., priming, docking, fusion, fusion pore opening, vesiculization and shedding. In mammals, fusion pore opening occurs at multiple sites over the acrosome, resulting in massive loss of the plasma membrane overlying the acrosome (shedding of the acrosomal cap).

Sperm-Egg Binding and Fusion

In most animals, the egg plasma membrane is surrounded by a firm membranous acellular structure collectively called the egg coat, or more routinely called vitelline membrane, vitelline coat, vitelline envelope, vitelline layer, chorion, and zona pellucida for specific animal groups. In representative species so far examined, the egg coat is consist of several (glyco)proteins as the major components. The vitelline membrane seems to have essential roles for recognition of spermatozoa from conspecific species to avoid interspecific hybridization, and protection of eggs from microorganisms, mechanical damages and multiple-sperm entry. A fertilizing spermatozoon can penetrate the egg coat at any location in most animal species with a few exceptions such as birds.

In sea urchins, the vitelline layer is very thin and bound tightly to the egg plasma membrane before fertilization. Acrosome-reacted spermatozoa adhere to the vitelline layer in a species-preferential manner via the acrosomal process. Such the species-specific adhesion is mediated by interaction between the complementary molecules called sperm bindin and its receptor, a high molecular weight glycoprotein (approximately 350-kDa) on the egg vitelline layer (Hirohashi *et al.*, 2008). Cross-fertilizations, therefore hybrid embryos, were readily observed when the vitelline membrane is removed by protease treatment, hence the vitelline membrane serves the latest and most potent barrier against interspecific hybridization in sea urchins.

In ascidians, hermaphroditism is common and the level of self-sterility varied. For instance, all species of the families Ascidiidae and Corellidae, and many species of Styela and Molgula are self-fertile, whereas *Ciona intestinalis* and *Halocynthia roretzi* are self-sterile. Such the self-sterility system is accounted for operating at the level of sperm-egg coat interaction because naked eggs exhibit self-fertility. The vitelline membrane of ascidian eggs is present apart from the egg plasma membrane, implying the least involvement of eggs themself in self/non-self recognition. Thus, the ascidian vitelline membrane is capable of discriminating the spermatozoa from not only foreign species but also same individuals. The molecules responsible for self/non-self recognition, which are easily removed from the vitelline membrane in acid seawater (such as pH 3), have been identified as v-Themis-A/B in *Ciona*. In the

current model, it is proposed that when s-Themis-A/B on the sperm surface recognizes self v-Themis-A/B on the vitelline membrane, an intracellular Ca^{2+} rise takes place in the sperm, resulting in the detachment of spermatozoa from the vitelline membrane (Sawada *et al.*, 2014).

Mammalian eggs are surrounded by a thick egg coat called the zona pellucida that contains three (ZP1-3 in mouse) or four (ZP1-4 in human) glycoproteins. It seems that among the glycoproteins, ZP2 is necessary for the binding of spermatozoa to the zona pellucida and that this phenomenon is relatively, but not absolutely, species-specific. Recent analyses using gene-knockout mice have, however, shown that the spermatozoa lacking the zona-binding ability are also able to fertilize eggs in the presence of zona pellucida (Okabe, 2014).

In most species of molluscs including abalones, a vitelline membrane also acts as a barrier against interspecific fertilization, which is regulated by species-specific interaction between a sperm acrosome-derived cationic protein called lysin and its receptor glycoprotein called VERL (vitelline envelope receptor for lysin). Abalone spermatozoa (1 μm in diameter) can make a hole (3 μm in diameter) in the vitelline membrane and penetrate through it. Non-enzymatic action of abalone lysin is presumed to be responsible for such fine regulation. In contrast, it is thought that proteases are used to dissolve the egg coat in deuterostomes such as sea urchins, ascidians, and mammals. In ascidian *Halocynthia*, it is reported that sperm penetration through the egg coat is mediated by the extracellular ubiquitin/proteasome system (UBS), i.e., upon sperm activation, spermatozoa release proteasome, ubiquitin-conjugating enzyme, ubiquitin and ATP into the surrounding seawater. A vitelline coat 70-kDa glycoprotein, HrVC70, which serves as the receptor for sperm binding is subjected to extracellular ubiquitination followed by degradation by the sperm proteasome during fertilization (Sawada *et al.*, 2014). In mammals, extracellular UBS is also suggested to operate during zona lysis. In spite of energetic studies, the proteases responsible for the penetration of zona pellucida have not yet been determined. Since the mouse spermatozoa recovered from the perivitelline space can penetrate through the zona pellucida again (Okabe, 2014), the proteases, if any, are considered to persist on the sperm surface without diffusing after acrosome reaction.

In some animals, a tough and elastic membrane, called chorion, prevents sperm penetration even before fertilization. Alternatively, sperm entry is facilitated by the narrow canal produced in a certain region of chorion, micropyle. A single funnel-shaped micropyle is located near the animal pole in most teleost fish and cephalopod eggs, whereas 4–12 micropyles are present at the animal pole region in the paddlefish *Polyodon spathula*. The number and location of micropyles in insect eggs vary significantly among species. In the fruit fly *Drosophila melanogaster*, one micropyle is present in a pointed protrusion at the anterior tip of the egg. In the grasshopper *Eyprepocnemis plorans*, an average of 40 micropyles are arranged in a ring-like manner close to the posterior pole. Queens of the termite *Reticulitermes speratus* can produce offspring both sexually and asexually (parthenogenetically) by controlling proportion of micropyleless eggs over time (Yashiro and Matsuura, 2014).

Eggs of many cnidarians including hydras, hydrozoan jellyfish, sea anemones, and corals, generally lack the firm egg coat, and the plasma membrane is covered with jelly layer. Nevertheless, site of sperm adhesion and subsequently site of sperm fusion are restricted to the plasma membrane of the animal pole just above the female pronucleus. In some species such as *Hydra* and *Hydractinia*, site of sperm-egg fusion appears as an indentation called the fertilization pit.

Monospermy and Physiological Polyspermy

In general, the entry of more than two spermatozoa into the egg cytoplasm, referred to as polyspermy, causes aberrant effects on meiosis completion or embryo development and hence embryonic death, due mainly to excess male centrosomes delivered into the egg. Thus, most animals have evolved multiple mechanisms by which monospermic fertilization is ensured (known for polyspermy block).

Changes in the electric potential of the egg plasma membrane are thought to play a central role in the fast block to polyspermy in various animals (Gould and Stephano, 2003). In sea urchin eggs, a resting membrane potential of approximately −70 mV shifts to +20 mV within a few seconds after the contact of a fertilizing spermatozoon. When Na^+ concentration is lowered in the surrounding water, this sperm-induced jump-up in the membrane potential, i.e., depolarization, does not occur, resulting in polyspermy. In addition, holding the membrane potential of unfertilized eggs at either $>+5$ or <-20 mV prevents sperm entry, whereas holding them between −20 and +5 mV allows repeated sperm entries. Thus, it is thought that the fertilizing spermatozoa can enter the egg within the narrow window of time during which Na^+-dependent depolarization takes place (or the egg becomes depolarized), ensuring monospermy at the initial phase of fertilization. However, it is a matter of debate whether or not the fast electrical polyspermy block is necessary under physiological conditions where not so many spermatozoa are assumed to reach the egg.

In many other marine invertebrates including ribbon worms, polychaete worms, echiuran worms, starfish, ascidians, bivalves, and gastropods, a similar depolarization from the resting negative potentials (between −80 and −10 mV) to the positive levels (between +10 and +60 mV) mainly due to Na^+ influx or Na^+ and Ca^{2+} influxes is also thought to play a role in the fast polyspermy block. In contrast, in eggs of the crab *Maja squinado*, fertilization induces K^+ efflux-mediated hyperpolarization (a decline of the membrane potential from −50 to −80 mV), which is accounted for polyspermy block. In eggs of freshwater lamprey and frog, fertilization evokes Cl^- efflux-mediated depolarization, resulting in polyspermy block.

In contrast to the aforementioned animals that show a fast electrical block to polyspermy, comb jellies, jellyfish, insects, teleost fish, newts, reptiles, birds, and mammals are regarded as devoid of such a system. Instead, in most teleost fish and various insects, the number of spermatozoa adhering to the egg in the initial phase of fertilization is limited in space and time by having a single or multiple micropyles on the chorion of an egg. Jellyfish eggs also ensure monospermic fertilization by restricting the site of sperm-egg fusion to the plasma membrane of the animal pole (5–10 μm in diameter).

After the successful entry of a spermatozoon, the fertilized egg generally exhibits a so-called late block to polyspermy, which includes physical and chemical modifications of the egg coat and the egg plasma membrane. In animals such as sea urchins, frogs, and mammals, secretion of the cortical granules results in the release of proteinous (e.g., proteases and ovoperoxidase) and chemical (e.g., H_2O_2) components to modify the egg coat (e.g., formation of the fertilization membrane in sea urchin eggs), which prevents the extra spermatozoa from adhering to or passing through it (Wessel and Wong, 2009). The cortical granule exocytosis in teleost fish eggs causes shrinkage of inner layer chorion, resulting in a decrease in the diameter of micropyle or the complete closure of this gate to abolish the following sperm entry (Murata, 2003). Late block to polyspermy at the level of plasma membrane independent of membrane potential has been reported in many animals including jellyfish, bivalves, and mammals. The loss of Juno (folate receptor 4), an egg receptor for the sperm ligand Izumo, is one of the reasons for the late plasma membrane block in mammalian eggs.

In contrast to monospermic fertilization that is absolutely necessary for eggs of many species to develop normally, there are some animals where polyspermy is mandatory for normal embryo development. This phenomenon, called physiological polyspermy, is found in vertebrates such as elasmobranchs (sharks and rays), urodeles (newts and salamanders), reptiles, and birds, as well as marine invertebrates such as comb jellies. In these animals, only a single sperm nucleus is selected to fuse with the egg nucleus, resulting in formation of a diploid zygotic nucleus. Consequently, other sperm nuclei and accompanying centrosomes are diminished without participating in the formation of fertilized egg (Iwao, 2012). Although monospermic fertilization is more common in insects, polyspermic fertilization is often seen in some species. Similarly, only a single male nucleus can unite with a female nucleus. This pattern is called compensable polyspermy (Snook *et al.*, 2011). Even in the species displaying physiological and compensable polyspermy, the time of fertilization is limited. For instance, fertilized avian eggs quickly lose their fertilizability due to the formation of chalaza-layer immediately after ovulation.

Egg Activation by Ca^{2+} Rise

Fertilized eggs exhibit a series of processes required for the release from cell cycle arrest, by which normal development can start, which is collectively called egg activation. The key event that leads to egg activation is a transient rise in the cytoplasmic Ca^{2+} concentration in fertilized eggs (from 100 nM to 2–3 μM in the case of sea urchins), which in turn modifies the activities of various Ca^{2+}-dependent proteins including protein kinases and phosphatases. Although it is known that eggs of all animal species investigated so far exhibit a Ca^{2+} rise at fertilization, there are considerably greater interspecific variations in the source of Ca^{2+}, the pattern of Ca^{2+} rises, and even the role of elevated Ca^{2+} (Kashir *et al.*, 2013).

In most animals, a fertilizing spermatozoon carries a substance(s) that triggers Ca^{2+} rise in the egg. In ribbon worms, ascidians, newts, birds, and mammals, it is thought that a sperm-borne soluble factor, called sperm factor, that evokes the Ca^{2+} rise is introduced into the egg cytoplasm after sperm-egg fusion. Recent analyses have shown that mammalian sperm factor corresponds to a sperm-specific phospholipase C (PLC) called PLC, which cleaves phosphatidylinositol 4,5-bisphosphate (PIP_2) into IP_3 and diacylglycerol in eggs. In newts, delivery of citrate synthase from spermatozoa is the main pathway to trigger the Ca^{2+} rise probably through elevation of PLC activity in eggs. In contrast to the internally acting soluble sperm factor, several lines of evidence indicate that many other animals including echiurans and frogs use the system where sperm protein binds to an egg receptor that links to intracellular pathways leading to a Ca^{2+} rise. In some animals such as *Drosophila*, prawns, and zebrafish, a Ca^{2+} rise occurs at the time of ovulation without stimulation by spermatozoa. Up-regulation of egg surface mechanosensitive ion channels that occurs in response to squeezing or swelling the egg would be responsible for the Ca^{2+} rise in *Drosophila*. Induction of Ca^{2+} rise without sperm would lead the way that eggs can also reproduce via parthenogenesis, which is frequently seen in arthropods and other animals.

Both external medium that contains Ca^{2+} (such as seawater) and intracellular organelles that store Ca^{2+} (such as the endoplasmic reticulum, ER) are the potential sources from which the Ca^{2+} are mobilized to the egg cytoplasm. Typically, a Ca^{2+} wave starts at the site of sperm-egg fusion and thereafter propagates the antipode (**Fig. 2(A)**), which is observed at fertilizations of such animals as jellyfish, echinoderms, ascidians, amphibians, and mammals. Polyspermic fertilization in newt eggs is accompanied by multiple Ca^{2+} waves that originate from respective sperm-egg fusion sites. Since each Ca^{2+} wave in newt eggs only propagates to one-eighth to one-quarter of the egg, multiple sperm entries are required to generate a Ca^{2+} rise over the entire egg. The Ca^{2+} waves initiated from a single or multiple points are mainly mediated by ER membrane-located IP_3 receptors that promote the Ca^{2+} release from the ER lumen. In contrast, a centripetal Ca^{2+} wave initiated at the whole cortex (**Fig. 2(B)**), which is probably dependent on Ca^{2+} influx through voltage-gated Ca^{2+} channels on the plasma membrane, is detected in fertilized eggs of ribbon worms, bivalves, limpets, and echiurans.

In jellyfish, some bivalves, limpets, echiurans, sea urchins, starfish, fish, and frogs, a single Ca^{2+} rise lasts for a few minutes to several tens of minutes (**Fig. 2(C)**). Fertilized eggs of ribbon worms, some bivalves, polychaetes, ascidians, and mammals, in contrast, exhibit repetitive Ca^{2+} rises, called Ca^{2+} oscillations, that persist for several tens of minutes to several hours (**Fig. 2(D)**).

In spite of the difference in the pattern and source of Ca^{2+}, one of the universal roles of the Ca^{2+} rise in the entire egg is to trigger the resumption of cell cycle; unfertilized eggs are arrested at either the first prophase (e.g., some bivalves and echiurans), the first metaphase (e.g., some bivalves, limpets, insects, and ascidians), the second metaphase of meiosis (e.g., most vertebrates), or the G_1 stage after completion of meiosis (e.g., jellyfish and sea urchins). It is known that Ca^{2+}-dependent proteins such as Ca^{2+}/calmodulin-dependent protein kinase II and calcineurin are involved in the downstream effector molecules responsible for the resumption of cell cycle in insects, ascidians, frogs, and mammals. A Ca^{2+} rise also modifies the physical and chemical properties of egg coat and plasma membrane through cortical granule exocytosis or other mechanisms to protect embryos as well as to prevent

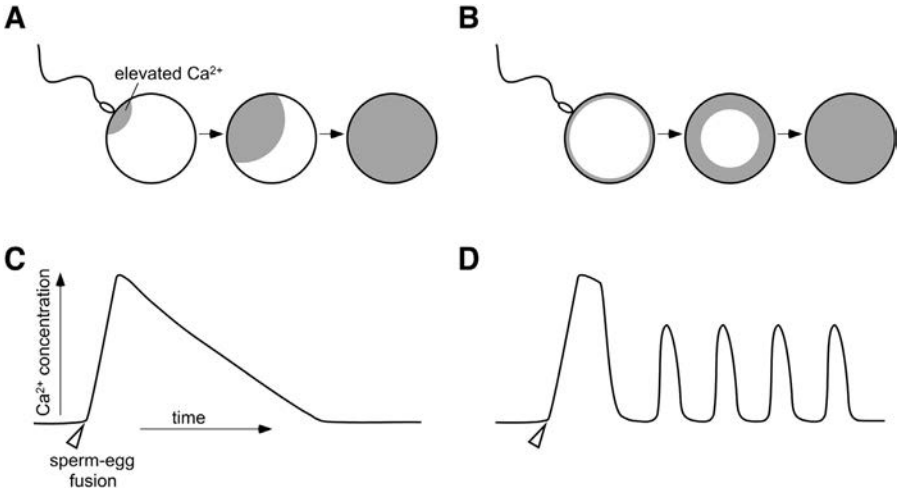


Fig. 2 Spatial and temporal patterns of intracellular Ca^{2+} rise in fertilized eggs. (A): Point-source Ca^{2+} wave initiated at the sperm-egg fusion site. (B): Centripetal Ca^{2+} wave initiated at the whole cortex. (C): Single Ca^{2+} rise. (D): Ca^{2+} oscillations.

polyspermy in the later phase. Ca^{2+} influx through voltage-dependent Ca^{2+} channels in many marine invertebrates and Cl^- efflux through Ca^{2+}-activated Cl^- channels in frogs contribute to the membrane depolarization that serves as fast electrical polyspermy block. It seems that the formation of cytoplasmic protrusion generated by a local Ca^{2+} rise helps the fertilizing spermatozoa on the vitelline envelope to enter the egg cytoplasm efficiently in some polychaetes, where unfertilized eggs have a wide perivitelline space (Nakano *et al.*, 2008). The pattern and amplitude of Ca^{2+} rise at fertilization also influence the later embryonic development (Whitaker, 2008). In ascidian and frog eggs, the direction of Ca^{2+} wave may specify the orientation of an embryonic axis. In mammals, abnormal patterns of Ca^{2+} oscillations are known to lower the developmental competence of blastocysts, resulting in fewer offspring.

References

Belmonte, S. A., Mayorga, L. S., & Tomes, C. N. (2016). The molecules of sperm exocytosis. *Advances in Anatomy, Embryology and Cell Biology, 220*, 71–92.

Birkhead, T. R., & Møller, A. P. (2008). Sexual selection and the temporal separation of reproductive events: Sperm storage data from reptiles, birds and mammals. *Biological Journal of the Linnean Society, 50*, 295–311.

Birkhead, T. R., & Pizzari, T. (2002). Postcopulatory sexual selection. *Nature Reviews Genetics, 3*, 262–273.

Gonzalez-Martinez, M. T., Galindo, B. E., de De La Torre, L., et al. (2001). A sustained increase in intracellular Ca^{2+} is required for the acrosome reaction in sea urchin sperm. *Developmental Biology, 236*, 220–229.

Gould, M. C., & Stephano, J. L. (2003). Polyspermy prevention in marine invertebrates. *Microscopy Research and Technique, 61*, 379–388.

Gunaratne, H. J., Moy, G. W., Kinukawa, M., et al. (2007). The 10 sea urchin receptor for egg jelly proteins (SpREJ) are members of the polycystic kidney disease-1 (PKD1) family. *BMC Genomics, 8*, 235.

Hirohashi, N., Kamei, N., Kubo, H., et al. (2008). Egg and sperm recognition systems during fertilization. *Development, Growth & Differentiation, 50*, S221–S238.

Iwao, Y. (2012). Egg activation in physiological polyspermy. *Reproduction, 144*, 11–22.

Kashir, J., Deguchi, R., Jones, C., Coward, K., & Stricker, S. A. (2013). Comparative biology of sperm factors and fertilization-induced calcium signals across the animal kingdom. *Molecular Reproduction and Development, 80*, 787–815.

La Spina, F. A., Puga Molina, L. C., Romarowski, A., et al. (2016). Mouse sperm begin to undergo acrosomal exocytosis in the upper isthmus of the oviduct. *Developmental Biology, 411*, 172–182.

Miki, K., & Clapham, D. E. (2013). Rheotaxis guides mammalian sperm. *Current Biology, 23*, 443–452.

Murata, K., 2003. Blocks to polyspermy in fish: A brief review. In: Aquaculture and Pathobiology of Crustacean and Other Species, Proceedings of the 32nd UJNR Aquaculture Panel Symposium, Davis and Santa Barbara, CA, pp. 1–15.

Nakano, T., Kyozuka, K., & Deguchi, R. (2008). Novel two-step Ca^{2+} increase and its mechanisms and functions at fertilization in oocytes of the annelidan worm *Pseudopotamilla occelata*. *Development, Growth & Differentiation, 50*, 365–379.

Okabe, M. (2014). Mechanism of fertilization: A modern view. *Experimental Animals, 63*, 357–365.

Sawada, H., Yamamoto, K., Otsuka, K., et al. (2014). Allorecognition and lysin systems during ascidian fertilization. In H. Sawada, N. Inoue, & M. Iwano (Eds.), *Sexual Reproduction in Animals and Plants* (pp. 231–244). Tokyo: Springer.

Snook, R. R., Hosken, D. J., & Karr, T. L. (2011). The biology and evolution of polyspermy: Insights from cellular and functional studies of sperm and centrosomal behavior in the fertilized egg. *Reproduction, 142*, 779–792.

Vacquier, V. D. (2012). The quest for the sea urchin egg receptor for sperm. *Biochemical Biophysical Research Communications, 425*, 583–587.

Vilela-Silva, A. C., Castro, M. O., Valente, A. P., Biermann, C. H., & Mourao, P. A. (2002). Sulfated fucans from the egg jellies of the closely related sea urchins *Strongylocentrotus droebachiensis* and *Strongylocentrotus pallidus* ensure species-specific fertilization. *The Journal of Biological Chemistry, 277*, 379–387.

Wessel, G. M., & Wong, J. L. (2009). Cell surface changes in the egg at fertilization. *Molecular Reproduction and Development, 76*, 942–953.

Whitaker, M. (2008). Calcium signalling in early embryos. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences, 363*, 1401–1418.

Yanagimachi, R., Harumi, T., Matsubara, H., et al. (2017). Chemical and physical guidance of fish spermatozoa into the egg through the micropyledagger, double dagger. *Biology of Reproduction, 96*, 780–799.

Yashiro, T., & Matsuura, K. (2014). Termite queens close the sperm gates of eggs to switch from sexual to asexual reproduction. *Proceedings of the National Academy of Sciences of the United States of America, 111*, 17212–17217.

Aspects of Rodent Implantation

Jeeyeon M Cha, Vanderbilt University Medical Center, Nashville, TN, United States
Wenbo Deng, Jia Yuan, and Sudhansu K Dey, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States

Introduction

Sexual reproduction and genetic diversification are foundations for evolutionary success to ensure perpetuation of the species by selecting for traits optimal for survival. Along this vein, mammalian pregnancy involves dynamic changes within the hormonally responsive pregnant uterus, nascent embryo, and transiently-lived mosaic tissues that promote their growth and reciprocal interactions. This involves the complex sequence of embryo development and implantation, decidualization, placentation and finally birth of offspring through the process of parturition (Dey et al., 2004; Lim and Wang, 2010; Cha et al., 2012). While the success of each event is critical for advancement to the next stage, the hierarchical directives that orchestrate embryo-uterine crosstalk are not fully characterized. These events are primarily directed by ovarian estrogen and progesterone (Dey et al., 2004); however, the molecular dialogue between the mother and embryo governing the orderly transitions of pregnancy events is yet to be fully understood.

The uterus is embryologically derived from the intermediate mesoderm and Mullerian ducts, which fuse at their caudal ends and differentiate to form the distinct cell types of the myometrium and the endometrium (Finn and Porter, 1975). The endometrium is hormonally responsive to ovarian hormones and comprised of the luminal epithelium, stroma and interspersed tubular glands which open and release contents into the uterine lumen. While the majority of endometrial cell types are defined during development, gland formation occurs postnatally from the uterine lumen as invaginations projecting toward the antimesometrial domain and is believed to be hormonally mediated (Filant and Spencer, 2014). A recent study shows that a differential Wnt gradient at the mesometrial (M) and anti-mesometrial (AM) domains directs the distribution of glands at the AM domain (Goad et al., 2017). Additional evidence shows that glands opening into the lumen secrete LIF under the regulation of FoxA2, which is essential for implantation in mice (Kelleher et al., 2017).

Molecular and cellular changes occur early in pregnancy to prepare the uterus for implantation (receptive phase). With the acquisition of blastocyst competency in the receptive uterus, implantation ensues (Dey et al., 2004). This "window of implantation" is a time-sensitive transient period when blastocyst competency coincides with the receptive state of the uterus on specific day(s) of pregnancy. When this coordination falls out of phase, implantation fails or becomes defective and generates adverse ripple effects throughout the course of pregnancy, leading to a poor pregnancy outcome (Song et al., 2002; Cha et al., 2012). The receptive window for implantation is also thought to be transient in humans; implantation past this window can lead to spontaneous miscarriages (Wilcox et al., 1999). In humans, the natural rate of conception per cycle (~30%), and up to 75% of failed pregnancies seem to be due to implantation failure (Norwitz et al., 2001). However, a significant number of miscarriages result from abnormal embryo development due to aneuploidy (reviewed in (Cha et al., 2012).

Implantation Stages and Types

Embryo implantation was historically dubbed "nidation" which originates from the word "nidus", meaning a nest or breeding place. It is the first cooperative physical and physiological interaction between the epithelium (trophectoderm) of the blastocyst and the maternal endometrial lining (luminal epithelium). The prerequisites for mammalian embryo implantation include preimplantation embryo development and blastocyst competency for implantation, differentiation of the uterus to a receptive state, formation of uterine crypts (implantation chambers) and attachment of the blastocyst with the luminal epithelium. These events are the result of a reciprocal blastocyst-uterine dialogue (Lim and Wang, 2010; Cha et al., 2012). In mice and rats, initiation of implantation occurs on the evening of day 4 (day 1 = morning of finding vaginal plug) or day 5 of pregnancy (day 1 = sperm in vaginal smears), respectively. One of the early signs of implantation in many species is a local increase in vascular permeability at the site of blastocyst apposition, which can be monitored by an intravenous injection of a macromolecular blue dye that binds to serum proteins and leaks out at sites of increased vascular permeability; this process demarcates the implantation sites as distinct blue bands (Psychoyos, 1973). Following implantation, the underlying stromal cells proliferate and differentiate to decidual cells (decidualization) accompanied by marked matrix remodeling and angiogenesis to establish communications between the nascent vascular system of the conceptus and mother to form the presumptive placenta (Cha et al., 2012).

Enders and Schlafke characterized implantation as occurring in three discrete stages: apposition, adhesion, and penetration (Enders and Schlafke, 1969). During apposition, the blastocyst trophectoderm becomes closely apposed to the uterine luminal epithelium (LE). When this intimate association is sufficient to resist dislocation of the blastocyst upon flushing the uterine lumen, implantation has progressed to the adhesion stage. Penetration is then associated with the breaching of LE by the trophectoderm. By this stage, decidualization of underlying stromal cells progresses with extensive loss of the epithelial cells.

The entry of trophoblast cells into the stroma with the advancement of implantation was thought to be mediated by epithelial apoptosis. However, recent studies in mice have provided evidence that epithelial cells in contact with the blastocyst are engulfed by trophectoderm cells by a nonapoptotic, cell-in-cell internalization process called entosis (Li et al., 2015). This process, which has

been previously characterized in cancer, was shown to have a physiologic role in normal pregnancy. However, the molecular mechanism behind this process is yet to be defined.

Implantation strategies vary between species and have been classified on the basis of divergent cell-cell interactions between the blastocyst and uterus. Bonnet classified implantation into three categories: central, eccentric and interstitial (Bonnet, 1884). Central implantation, observed in rabbits, ferrets, and some marsupials, occurs when blastocysts extensively expand prior to implantation to be closely apposed and maximally interact with the LE. In contrast, blastocysts in mice, rats, and hamsters show only modest expansion and undergo eccentric implantation, during which implantation chambers are formed within evaginations from the uterine lumen. Notably in rodents, implantation always occurs at the anti-mesometrial (AM) domain of the uterus, opposite to the mesometrial (M) domain, the entry site of blood vessels into the uterus (see below: **Uterine Changes in Implantation**). In contrast, implantation occurs at the mesometrial site in bats. Interestingly, during interstitial implantation observed in guinea pigs, chimpanzees and humans, blastocysts implant by entrenching into the subepithelial stroma (Dey et al., 2004).

Schlafke and Enders further classified implantation types as intrusive, displacement and fusion based on the results of ultrastructural electron microscopy studies (Schlafke and Enders, 1975). During intrusive types of implantation, which is seen in humans and guinea pigs, trophoblast cells penetrate through the LE to reach the basal lamina. In displacement type of implantation which occurs in rodents, the basal lamina disassociates from the overlying LE to facilitate trophoblast invasion into the subepithelial stromal bed. In contrast, rabbits exhibit fusion type of implantation in which trophoblast cells fuse with the LE by forming symplasma (trophoblastic knob, the syncytial aggregates that attach to and invade the endometrium) (Schlafke and Enders, 1975). In rabbits, attachment stimulates an angiogenic response, particularly at the trophoblastic knobs, the syncytial aggregates that attach to and invade the endometrium with increased expression of vascular endothelial growth factor (Das et al., 1997).

Noninvasive implantation has been observed in large animals and marsupials, such as the pig, sheep, cow, horse, and wallaby (Renfree and Shaw, 2000; Roberts et al., 2008; Bazer, 2015). For example, pig blastocysts remain in a "free-floating" state with noninvasive implantation until day 12 (termed pregnancy recognition) at which point it elongates up to 100 mm in length, primarily due to the rapid growth of the extraembryonic tissue. This strategy allows an efficient nutrient and metabolite exchanges between the uterus and conceptus until the attachment reaction.

Uterine Changes During Implantation

Corner once remarked: "the uterine chamber is actually a less favorable place for early embryos to implant than say, the anterior chamber of the eye, except when the hormones of the ovary act upon it and change it into a place of superior efficiency for its new functions" (Corner, 1947). The differentiation of the uterus to a receptive state during pregnancy that renders it favorable for the implantation and development of an embryo was first described by Alexandre Psychoyos. By reciprocal embryo transfer experiments using pseudopregnant and delayed-implanting rodent models, he established the concept of the transient window of receptivity by showing that blastocysts only implanted when transferred into a hormonally prepared, receptive uterus (Psychoyos, 1973). It was found that blastocysts implantation in uteri of mice or rats requires at least 24–48 h of P_4 priming superimposed with a small amount of estrogen (Psychoyos, 1973). Later, reciprocal blastocyst transfer experiments showed that the acquisition of blastocyst activation and uterine receptivity for implantation both require in vivo exposure to estrogen (Paria et al., 1993). Thus far, all mammals studied exhibit a transient window of uterine receptivity of varying duration for implantation (Cha et al., 2012; Yoshinaga, 2013).

Although both P_4 and estrogen are essential for implantation in mice and rats, ovarian estrogen is not a requirement for implantation in hamsters, rabbits and pigs. Blastocysts can implant in these species only in the presence of P_4; surprisingly, neither P_4 nor estrogen is required for implantation in guinea pigs (Deanesly, 1963). It was conjectured that embryos in these species can locally produce the steroid hormones required for implantation. Indeed, biochemical experiments provided evidence that the rabbit and pig blastocysts have the capacity to synthesize estrogens (Perry et al., 1973; Hoversland et al., 1982); this capacity was not found in mouse blastocysts (Stromstedt et al., 1996).

The acquisition of uterine receptivity in preparation for blastocyst attachment is reflected in both cellular and molecular changes involving the three major uterine compartments (epithelium, stroma, and myometrium) uniquely responding to changing ovarian P_4 and estrogen secretion. To create the window of uterine receptivity, the cooperative interactions between P_4 and estrogen regulate uterine cell proliferation and/or differentiation in a spatiotemporal manner in mice and rats. In rodents and humans, there is a gradual loss of apicobasal LE cell polarity and formation of microprotrusions called pinopodes or uterodomes on the apical surface of the LE impending blastocyst attachment (Thie et al., 1996; Nikas and Psychoyos, 1997).

Along the same vein, the epithelial apico-basal cell polarity is considered critical to implantation. In the search for the molecular mechanism underpinning this event, muscle-segment homeobox genes *Msx1* and *Msx2* were found to play a major role in modulating apico-basal polarity and implantation by altering uterine *Wnt5a* levels (Daikoku et al., 2011). MSX transcription factors are also important for regulating delayed implantation in the mouse, tammar wallaby and North American mink (Cha et al., 2013b). The molecular aspects of embryo competency and uterine receptivity are described in separate chapters.

Blood vessels travel to the uterus through the mesometrium and anatomically orient the uterus into mesometrial and antimesometrial domains (Cha et al., 2012). In rodents, embryo homing and implantation occur within a crypt (implantation

chamber) invariably at the AM domain along the uterus. On day four of pregnancy in mice (day 1 = vaginal plug), epithelial evaginations (folding) project from the uterine lumen toward the AM domain. A certain number of projections provokes blastocysts to form crypts for embryo implantation. The unique architectural organization of crypts for blastocyst homing and attachment was first recognized in rodents more than a century ago (Burckhard, 1901) and later by Enders' group (Enders et al., 1980). However, the mechanism by which epithelial evaginations are appropriately directed to form crypts at the AM domain for embryo homing and implantation remained unknown. It is also remains undefined how the crypts homing the embryos are regularly spaced.

Recent studies show that crypt formation at the AM domain requires non-canonical Wnt5a-ROR signaling. Epithelial projections form along a *Wnt5a* expression gradient, and mice with disruption of uterine Wnt5a-ROR signaling with conditional uterine loss or gain of function of Wnt5a or loss of function of *Ror1*/*Ror2* (Wnt5a co-receptors) resulted in disorderly epithelial projections, crypt formation, embryo spacing, and impaired implantation (Cha et al., 2014). These early disturbances under abnormal Wnt5a-ROR signaling were reflected in adverse late pregnancy events (Cha et al., 2014). Further studies showed that Wnt5a-ROR signaling intersects planar cell polarity (PCP) signaling orchestrating the formation of directed epithelial evaginations to form crypts for implantation in mice (Yuan et al., 2016).

PCP is a developmental pathway essential for establishing spatial cues in multicellular tissues during organogenesis and branching morphogenesis during mammary gland development (Yang and Mlodzik, 2015). In animal models, disrupting PCP signaling genetically or pharmacologically results in abnormal hair cell orientation, giving rise to defects in neural tube closure and left-right asymmetry; in humans, polymorphisms of PCP components are associated with an array of developmental defects including spina bifida and cardiac outflow malformations (van Amerongen and Nusse, 2009). While PCP has been studied extensively during development, its role in adult tissues in physiological conditions has been limited. Recent studies show that uterine deletion of Vang-like protein 2 (Vangl2), but not Vangl1, confers aberrant PCP signaling, misdirected epithelial evaginations, defective crypt formation, and blastocyst attachment, leading to compromised pregnancy outcomes (Yuan et al., 2016). The study identified a novel role for PCP in executing spatial cues for crypt formation and implantation (Fig. 1).

PCP signaling is evolutionarily conserved, although it is not known whether uterine PCP activity is important for implantation across species including humans. Additional studies have shown that the correct orientation of the embryonic axis within the crypt is also critical for pregnancy success in mice (Zhang et al., 2014); Notch signaling was determined to be a key component.

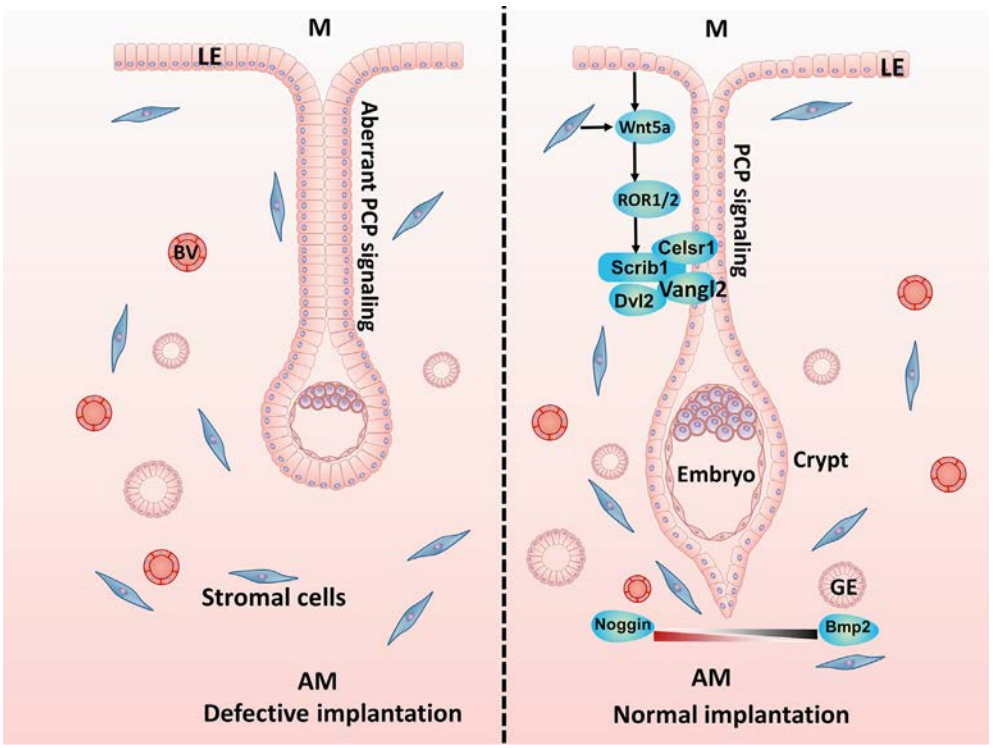


Fig. 1 A schematic diagram of PCP signaling pathway in designing epithelial evagination and crypt formation for embryo implantation at the AM domain of the uterus. As the downstream signaling of Wnt5a–ROR, Vangl2 directly interacts with Scrib, Celsr1, and Dvl2 to form a complex to maintain epithelium planar polarity. After blastocyst attachment within the crypt epithelium, an opposing balance between Noggin and Bmp2 signaling in the subepithelial stroma facilitates the progression of implantation and decidualization. Right panel shows Wnt5a–ROR–PCP signaling regulates the luminal epithelium to form a "conch-shaped" crypt to home the embryo (normal implantation). Left panel shows defective implantation in a roundish crypt due to aberrant PCP signaling. M, mesometrium; AM, antimesometrium; Bv, blood vessel; Ge, glandular epithelium; LE, luminal epithelium.

Defective Implantation and Decidualization Causes Adverse Ripple Effects

Despite scientific advances in human fertility by assisted reproductive technologies, the implantation rate and number of 'take-home' babies still remain low (Norwitz et al., 2001). Poor embryo quality, transfer of embryos into uteri of unknown states of receptivity, and complications from inferior quality of implantation are all barriers to successful pregnancy. Indeed, defective implantation was shown to propagate adverse ripple effects throughout the remainder of pregnancy, resulting in compromised pregnancy outcomes, including preterm birth and preeclampsia which can have life-long health effects on the offspring.

Mouse models have helped to define propagation of early defects through the remaining course of pregnancy. The first study of adverse ripple effects from defective implantation was noted in mice deficient in *Pla2g4a* (encoding cPLA2α), which generates arachidonic acid from membrane phospholipids for prostaglandin synthesis via COX enzymes. Implantation in *Pla2g4a* −/− mice occurs beyond the normal window of implantation (deferred implantation), resulting in embryo crowding, conjoined placenta, arrested fetoplacental development, increased resorptions and reduced litter size (Song et al., 2002). The authors also performed proof-of-principle physiological experiments in which blastocysts were transferred to wild-type mouse uteri beyond the anticipated window of uterine receptivity and showed similar adverse phenotypes of reduced litter size and increased resorption. Finally, similar findings were observed for mice deficient in Lpar3 (LPA3), a receptor for lysophosphatidic acid (Ye et al., 2005). The similar phenotypes were attributed to reduced production of prostaglandins generated by COX2, implicating a LPA3-cPLA$_2$-Cox2 signaling axis as a critical determinant for on-time implantation.

Preeclampsia is believed to result from abnormal placentation. A recent study in a mouse model which spontaneously develops the cardinal features of preeclampsia showed periimplantation defects including upregulation of Cox2 and IL-15 at the maternal-fetal interface (Sones et al., 2016). This was associated with decreased decidual natural killer (dNK) cells, which have important roles in placental perfusion. A single administration of a Cox2 inhibitor (celecoxib) during decidualization restrained Cox2 activity and IL-15 expression, restored dNK cell numbers, improved fetal growth, and attenuated late gestational hypertension. This study provides evidence that decidual overexpression of Cox2 and IL-15 may trigger the adverse pregnancy outcome reflected in the preeclampsia syndrome.

Aberrant decidualization can also give rise to adverse pregnancy phenotypes including aberrations in parturition. Preterm birth is one example of an adverse pregnancy effect of defective decidualization. The Dey lab has shown that mice with uterine deletion of *Trp53* (encoding p53; *Trp53*$^{d/d}$) show normal implantation, however, 50–60% of *Trp53*d/d mice exhibit preterm birth with dystocia and fetal death (Hirota et al., 2010). These mice have compromised decidualization with more terminally differentiated decidual cells than control littermates with increased polyploidy, senescence-associated growth restriction, and heightened expression of pAkt, p21 and Cox2. Many risk factors, such as genetic mutation, infection, inflammation and stress that lead to preterm birth are also reported to exacerbate cellular senescence via mammalian target of rapamycin complex 1 (mTORC1) signaling. Rapamycin attenuates senescence and increase life span in mice (Harrison et al., 2009). *Trp53*d/d decidua have increased mTORC1 activity, which is inhibited by rapamycin or metformin with attenuation of premature decidual senescence and rescue of preterm birth (Hirota et al., 2010; Cha et al., 2013a). This finding is intriguing since women of advanced age exhibit higher rates of preterm birth (Cnattingius et al., 1992) and a cohort of Japanese women who delivered preterm showed increased decidual senescence compared to term counterparts (Cha et al., 2013a). These results are a paradigm shift in our understanding of the physiology of birth timing and pathogenesis of preterm birth by identifying a decidual origin of preterm birth which can be targeted using mTORC1 inhibitors (**Fig. 2**). Whether this intervention can be applied broadly remains to be studied; clinical research to study decidual senescence in women with higher risk factors for preterm birth will be useful to target this global problem.

In this regard, higher levels of endocannabinoid signaling have also been shown to enhance preterm birth in response to a mild inflammatory stimulus. Increased p38/MAPK independent of mTORC1 signaling accelerates decidual senescence (Sun et al., 2016), suggesting that decidual senescence may function as a common final pathway integrating multiple signaling pathways toward birth timing.

Future Considerations

With marked advances in technology, much remains to be revealed about the dynamic physiological and molecular interactions encompassing early pregnancy events. The precise timing of implantation and the molecular discourse between the embryo and uterus have yet to be determined in humans. Already, advances in high fidelity RNA−Seq and MALDI-MS proteomics have helped to identify low-abundance molecular and modified mediators not previously noted. Recent application of in situ mass spectrometry in periimplantation mouse uteri also provide opportunities to generate spatiotemporal maps of proteins and lipids as well as their modifications in other species including humans (Burnum et al., 2009). Furthermore, analysis of the single cell transcriptome of human preimplantation embryos and the epigenetic signature of primordial germ cells has opened new avenues to map the transcriptome of single epithelial and stromal cells in the uterus around the time of implantation (Yan et al., 2013; Guo et al., 2015; Petropoulos et al., 2016). These datasets coupled with those from MALDI-MS proteomics and lipidomics profiles can be used to

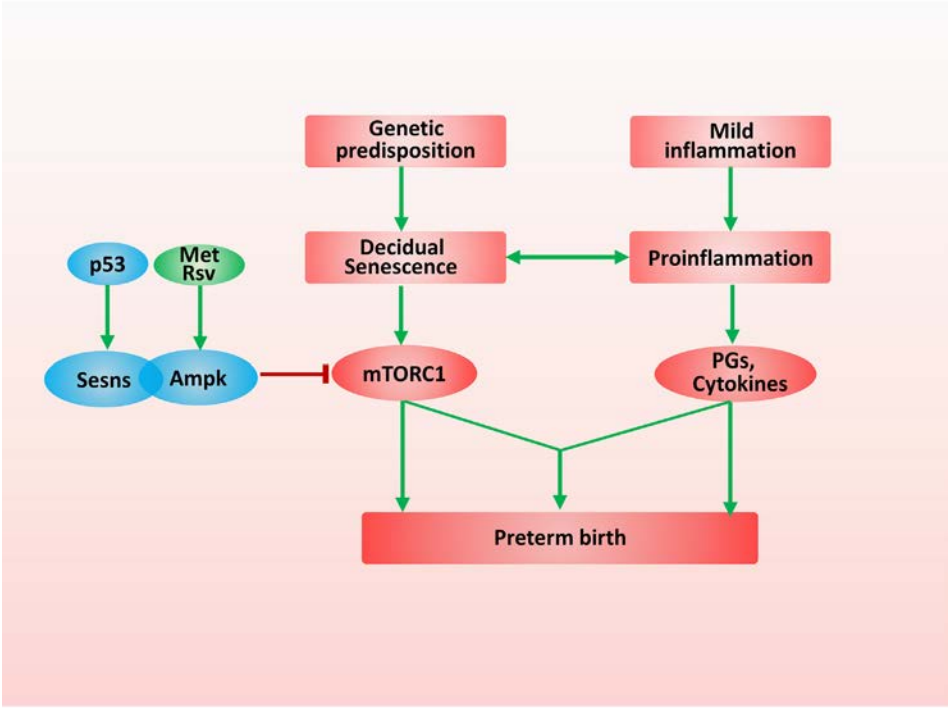


Fig. 2 A scheme showing that defective decidualization leads to preterm birth. Decidual senescence caused by genetic predisposition due to gene mutation superimposed by mild inflammation confers compromised decidual health and increased susceptibility to preterm birth. Heightened mTORC1 signaling is a signature of premature decidual. Signaling by p53-Sesn2 dampens mTORC1 signaling which is further helped by Met and Rsv through increased AMPK signaling in the context of inflammatory insult.

identify mediators secreted by embryos freely or in exosomes and their downstream effects that can promote their own growth or signal the endometrium for implantation.

Epigenetic regulation allows for versatile and reversible changes in gene expression without changing the underlying DNA sequence. New insights on epigenetic regulation of reproductive events continue to be gleaned, including the roles of histone modifications, chromatin remodeling, DNA methylation, microRNA and long noncoding RNAs (lncRNA), and novel transcription factor response elements such as the super enhancer identification (Pott and Lieb, 2015; Dekker and Mirny, 2016). The signature of estrogen and progesterone receptors binding sites and their downstream mediators have been assessed by genome-wide assays and are likely to have a substantial impact on implantation and pregnancy events (Pott and Lieb, 2015; Rubel et al., 2016; Vasquez et al., 2016). There is evidence regarding the roles of histone modifications in reproduction, especially the roles of the Polycomb repressive complexes 1 and 2 (PRC1 and PRC2) which are distinguishable by their core components. PRC1 components, including the E3 protein ligases responsible for ubiquitination of histone H2A, Ring1A and Ring1B, (Margueron and Reinberg, 2011) and chromobox (CBX) family (Schwartz and Pirrotta, 2013) are differentially and spatiotemporally expressed in the periimplantation uteri. Notably, inhibition of PRC1 activity by a Ring1A/B inhibitor compromises decidualization and polyploidy development during early pregnancy in vivo, while interference of Cbx4 expression in stromal cells also show defective stromal cell decidualization and polyploidy development in vitro (Bian et al., 2016). During decidualization and decidual polyploidy, DNA methylation was shown to be important in hormone-dependent gene expression in the pregnant uterus (Gao et al., 2015). H3K27 methyltransferase activity by PRC2 is relayed by its component proteins including EZH1/EZH2, SUZ12 polycomb repressive complex 2 subunit (SUZ12) and RB binding protein 7 (RBAP46) or RB binding protein 4 (RBAP48) (Blackledge et al., 2015). The increased H3K27me3 at the promoter of chemokine (C−C motif) ligand 8 (CCL8) and chemokine (C−C motif) ligand 9 (CCL9), critical chemokines for T cell migration from stroma to myometrium, confers a local immune-privileged region for embryo development, indicating the importance of this epigenetic mark in pregnancy (Nancy et al., 2012). Finally, EZH2 has dynamic expression during the menstrual cycle in humans and is suppressed in decidualized stromal cells, implicating a role for EZH2-PRC2 mediated chromatin remodeling in the human endometrium (Grimaldi et al., 2012). The role of these epigenetic marks in pregnancy events remains to be validated in a physiological setting. The novel Crispr-Cas9 system will provide an efficient way to systematically isolate the function of these targets in mammalian systems (Hsu et al., 2014; Gorski et al., 2017). Progress in precisely defining the molecular window of implantation and identifying regulators of dynamic embryo-uterine interactions will continue in stride with refined technological tools. These evolving areas of research warrant careful investigation in animal models which can then be expanded to human studies.

Acknowledgements

Our sincere appreciation to Katie Gerhardt for her help in editing this review article. The work described from Dey lab has been funded by NIH (R01HD068524 and DA006668) and March of Dimes (21-FY12-127 and 22-FY13-543). We regret that space limitations precluded us from citing many relevant references.

References

Bazer, F. W. (2015). History of maternal recognition of pregnancy. *Advances in Anatomy, Embryology, and Cell Biology, 216*, 5–25.

Bian, F., Gao, F., Kartashov, A. V., Jegga, A. G., Barski, A., & Das, S. K. (2016). Polycomb repressive complex 1 controls uterine decidualization. *Scientific Reports, 6*, 26061.

Blackledge, N. P., Rose, N. R., & Klose, R. J. (2015). Targeting Polycomb systems to regulate gene expression: Modifications to a complex story. *Nature Reviews. Molecular Cell Biology, 16*(11), 643–649.

Bonnet, R. (1884). Beitrage zur embryologie der wiederkauer, gewonnen am schafei. *Archiv für anatomie und physiologie, 8*, 170–230.

Burnum, K. E., Cornett, D. S., Puolitaival, S. M., Milne, S. B., Myers, D. S., Tranguch, S., Brown, H. A., Dey, S. K., & Caprioli, R. M. (2009). Spatial and temporal alterations of phospholipids determined by mass spectrometry during mouse embryo implantation. *Journal of Lipid Research, 50*(11), 2290–2298.

Cha, J., Bartos, A., Egashira, M., Haraguchi, H., Saito-Fujita, T., Leishman, E., Bradshaw, H., Dey, S. K., & Hirota, Y. (2013a). Combinatory approaches prevent preterm birth profoundly exacerbated by gene-environment interactions. *The Journal of Clinical Investigation, 123*(129), 4063–4075.

Cha, J., Bartos, A., Park, C., Sun, X., Li, Y., Cha, S. W., Ajima, R., Ho, H. Y., Yamaguchi, T. P., & Dey, S. K. (2014). Appropriate crypt formation in the uterus for embryo homing and implantation requires Wnt5a-ROR signaling. *Cell Reports, 8*(2), 382–392.

Cha, J., Sun, X., Bartos, A., Fenelon, J., Lefevre, P., Daikoku, T., Shaw, G., Maxson, R., Murphy, B. D., Renfree, M. B., & Dey, S. K. (2013b). A new role for muscle segment homeobox genes in mammalian embryonic diapause. *Open Biology, 3*(4).

Cha, J., Sun, X., & Dey, S. K. (2012). Mechanisms of implantation: Strategies for successful pregnancy. *Nature Medicine, 18*(12), 1754–1767.

Cnattingius, S., Forman, M. R., Berendes, H. W., & Isotalo, L. (1992). Delayed childbearing and risk of adverse perinatal outcome. A population-based study. *JAMA, 268*(7), 886–890.

Corner, G. (1947). *The hormones in human reproduction*. Princeton, NJ: Princeton University Press.

Daikoku, T., Cha, J., Sun, X., Tranguch, S., Xie, H., Fujita, T., Hirota, Y., Lydon, J., DeMayo, F., Maxson, R., & Dey, S. K. (2011). Conditional deletion of Msx homeobox genes in the uterus inhibits blastocyst implantation by altering uterine receptivity. *Developmental Cell, 21*(6), 1014–1025.

Das, S. K., Chakraborty, I., Wang, J., Dey, S. K., & Hoffman, L. H. (1997). Expression of vascular endothelial growth factor (VEGF) and VEGF-receptor messenger ribonucleic acids in the peri-implantation rabbit uterus. *Biology of Reproduction, 56*(6), 1390–1399.

Deanesly, R. (1963). Further observations on the effects of oestradiol on tubal eggs and implantation in the guinea-pig. *Journal of Reproduction and Fertility, 5*, 49–57.

Dekker, J., & Mirny, L. (2016). The 3D genome as moderator of chromosomal communication. *Cell, 164*(6), 1110–1121.

Dey, S. K., Lim, H., Das, S. K., Reese, J., Paria, B. C., Daikoku, T., & Wang, H. (2004). Molecular cues to implantation. *Endocrine Reviews, 25*(3), 341–373.

Enders, A. C., & Schlafke, S. (1969). Cytological aspects of trophoblast-uterine interaction in early implantation. *The American Journal of Anatomy, 125*(1), 1–29.

Enders, A. C., Schlafke, S., & Welsh, A. O. (1980). Trophoblastic and uterine luminal epithelial surfaces at the time of blastocyst adhesion in the rat. *The American Journal of Anatomy, 159*(1), 59–72.

Filant, J., & Spencer, T. E. (2014). Uterine glands: Biological roles in conceptus implantation, uterine receptivity and decidualization. *The International Journal of Developmental Biology, 58*(2–4), 107–116.

Finn, C., & Porter, D. (1975). *The Uterus*. London: Paul Elek (Scientific Books) Ltd.

Gao, L., Rabbitt, E. H., Condon, J. C., Renthal, N. E., Johnston, J. M., Mitsche, M. A., Chambon, P., Xu, J., O'Malley, B. W., & Mendelson, C. R. (2015). Steroid receptor coactivators 1 and 2 mediate fetal-to-maternal signaling that initiates parturition. *The Journal of Clinical Investigation, 125*(7), 2808–2824.

Goad, J., Ko, Y. A., Kumar, M., Syed, S. M., & Tanwar, P. S. (2017). Differential Wnt signaling activity limits epithelial gland development to the anti-mesometrial side of the mouse uterus. *Developmental Biology, 423*(2), 138–151.

Gorski, S. A., Vogel, J., & Doudna, J. A. (2017). RNA-based recognition and targeting: Sowing the seeds of specificity. *Nature Reviews. Molecular Cell Biology.*

Grimaldi, G., Christian, M., Quenby, S., & Brosens, J. J. (2012). Expression of epigenetic effectors in decidualizing human endometrial stromal cells. *Molecular Human Reproduction, 18*(9), 451–458.

Guo, F., Yan, L., Guo, H., Li, L., Hu, B., Zhao, Y., Yong, J., Hu, Y., Wang, X., Wei, Y., Wang, W., Li, R., Yan, J., Zhi, X., Zhang, Y., Jin, H., Zhang, W., Hou, Y., Zhu, P., Li, J., Zhang, L., Liu, S., Ren, Y., Zhu, X., Wen, L., Gao, Y. Q., Tang, F., & Qiao, J. (2015). The Transcriptome and DNA Methylome landscapes of human primordial germ cells. *Cell, 161*(6), 1437–1452.

Harrison, D. E., Strong, R., Sharp, Z. D., Nelson, J. F., Astle, C. M., Flurkey, K., Nadon, N. L., Wilkinson, J. E., Frenkel, K., Carter, C. S., Pahor, M., Javors, M. A., Fernandez, E., & Miller, R. A. (2009). Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature, 460*(7253), 392–395.

Hirota, Y., Daikoku, T., Tranguch, S., Xie, H., Bradshaw, H., & Dey, S. K. (2010). Uterine-specific p53 deficiency confers premature uterine senescence and promotes preterm birth in mice. *Journal of Clinical Investigation, 120*(3), 803–815.

Hoversland, R. C., Dey, S. K., & Johnson, D. C. (1982). Catechol estradiol induced implantation in the mouse. *Life Sciences, 30*(21), 1801–1804.

Hsu, P. D., Lander, E. S., & Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. *Cell, 157*(6), 1262–1278.

Kelleher, A. M., Peng, W., Pru, J. K., Pru, C. A., DeMayo, F. J., & Spencer, T. E. (2017). Forkhead box a2 (FOXA2) is essential for uterine function and fertility. *Proceedings of the National Academy of Sciences of the United States of America, 114*(6), E1018–E1026.

Li, Y., Sun, X., & Dey, S. K. (2015). Entosis allows timely elimination of the luminal epithelial barrier for embryo implantation. *Cell Reports, 11*(3), 358–365.

Lim, H. J., & Wang, H. (2010). Uterine disorders and pregnancy complications: Insights from mouse models. *The Journal of Clinical Investigation, 120*(4), 1004–1015.

Margueron, R., & Reinberg, D. (2011). The Polycomb complex PRC2 and its mark in life. *Nature, 469*(7330), 343–349.

Nancy, P., Tagliani, E., Tay, C. S., Asp, P., Levy, D. E., & Erlebacher, A. (2012). Chemokine gene silencing in decidual stromal cells limits T cell access to the maternal-fetal interface. *Science, 336*(6086), 1317–1321.

Nikas, G., & Psychoyos, A. (1997). Uterine pinopodes in peri-implantation human endometrium. Clinical relevance. *Annals of the New York Academy of Sciences, 816*, 129–142.

Norwitz, E. R., Schust, D. J., & Fisher, S. J. (2001). Implantation and the survival of early pregnancy. *The New England Journal of Medicine, 345*(19), 1400–1408.

Paria, B. C., Huet-Hudson, Y. M., & Dey, S. K. (1993). Blastocyst's State of activity determines the "window" of implantation in the receptive mouse uterus. *Proceedings of the National Academy of Sciences of the United States of America, 90*(21), 10159–10162.

Perry, J. S., Heap, R. B., & Amoroso, E. C. (1973). Steroid hormone production by pig blastocysts. *Nature, 245*(5419), 45–47.

Petropoulos, S., Edsgard, D., Reinius, B., Deng, Q., Panula, S. P., Codeluppi, S., Reyes, A. P., Linnarsson, S., Sandberg, R., & Lanner, F. (2016). Single-cell RNA-Seq reveals lineage and X chromosome dynamics in human Preimplantation embryos. *Cell, 167*(1), 285.

Pott, S., & Lieb, J. D. (2015). What are super-enhancers? *Nature Genetics, 47*(1), 8–12.

Psychoyos, A. (1973). Hormonal control of ovoimplantation. *Vitamins and Hormones, 31*, 201–256.

Renfree, M. B., & Shaw, G. (2000). Diapause. *Annual Review of Physiology, 62*, 353–375.
Roberts, R. M., Chen, Y., Ezashi, T., & Walker, A. M. (2008). Interferons and the maternal-conceptus dialog in mammals. *Seminars in Cell & Developmental Biology, 19*(2), 170–177.
Rubel, C. A., Wu, S. P., Lin, L., Wang, T., Lanz, R. B., Li, X., Kommagani, R., Franco, H. L., Camper, S. A., Tong, Q., Jeong, J. W., Lydon, J. P., & DeMayo, F. J. (2016). A Gata2-dependent transcription network regulates uterine progesterone responsiveness and endometrial function. *Cell Reports, 17*(5), 1414–1425.
Schlafke, S., & Enders, A. C. (1975). Cellular basis of interaction between trophoblast and uterus at implantation. *Biology of Reproduction, 12*(1), 41–65.
Schwartz, Y. B., & Pirrotta, V. (2013). A new world of Polycombs: Unexpected partnerships and emerging functions. *Nature Reviews. Genetics, 14*(12), 853–864.
Sones, J. L., Cha, J., Woods, A. K., Bartos, A., Heyward, C. Y., Lob, H. E., Isroff, C. E., Butler, S. D., Shapiro, S. E., Dey, S. K., & Davisson, R. L. (2016). Decidual Cox2 inhibition improves fetal and maternal outcomes in a preeclampsia-like mouse model. *Journal of Clinical Investigation Insight, 1*(3).
Song, H., Lim, H., Paria, B. C., Matsumoto, H., Swift, L. L., Morrow, J., Bonventre, J. V., & Dey, S. K. (2002). Cytosolic phospholipase A2alpha is crucial [correction of A2alpha deficiency is crucial] for 'on-time' embryo implantation that directs subsequent development. *Development, 129*(12), 2879–2889.
Stromstedt, M., Keeney, D. S., Waterman, M. R., Paria, B. C., Conley, A. J., & Dey, S. K. (1996). Preimplantation mouse blastocysts fail to express CYP genes required for estrogen biosynthesis. *Molecular Reproduction and Development, 43*(4), 428–436.
Sun, X., Deng, W., Li, Y., Tang, S., Leishman, E., Bradshaw, H. B., & Dey, S. K. (2016). Sustained Endocannabinoid signaling compromises Decidual function and promotes inflammation-induced preterm birth. *The Journal of Biological Chemistry, 291*(15), 8231–8240.
Thie, M., Fuchs, P., & Denker, H. W. (1996). Epithelial cell polarity and embryo implantation in mammals. *The International Journal of Developmental Biology, 40*(1), 389–393.
van Amerongen, R. and Nusse, R. (2009) Towards an integrated view of Wnt signaling in development. Development, 136(19). 3205–3214.
Vasquez, Y. M., Wu, S. P., Anderson, M. L., Hawkins, S. M., Creighton, C. J., Ray, M., Tsai, S. Y., Tsai, M. J., Lydon, J. P., & DeMayo, F. J. (2016). Endometrial expression of Steroidogenic factor 1 promotes cystic glandular morphogenesis. *Molecular Endocrinology, 30*(5), 518–532.
Wilcox, A. J., Baird, D. D., & Weinberg, C. R. (1999). Time of implantation of the conceptus and loss of pregnancy. *The New England Journal of Medicine, 340*(23), 1796–1799.
Yan, L., Yang, M., Guo, H., Yang, L., Wu, J., Li, R., Liu, P., Lian, Y., Zheng, X., Yan, J., Huang, J., Li, M., Wu, X., Wen, L., Lao, K., Li, R., Qiao, J., & Tang, F. (2013). Single-cell RNA-Seq profiling of human preimplantation embryos and embryonic stem cells. *Nature Structural & Molecular Biology, 20*(9), 1131–1139.
Yang, Y., & Mlodzik, M. (2015). Wnt-frizzled/planar cell polarity signaling: Cellular orientation by facing the wind (Wnt). *Annual Review of Cell and Developmental Biology, 31*, 623–646.
Ye, X., Hama, K., Contos, J. J., Anliker, B., Inoue, A., Skinner, M. K., Suzuki, H., Amano, T., Kennedy, G., Arai, H., Aoki, J., & Chun, J. (2005). LPA3-Mediated lysophosphatidic acid signalling in embryo implantation and spacing. *Nature, 435*(7038), 104–108.
Yoshinaga, K. (2013). A sequence of events in the uterus prior to implantation in the mouse. *Journal of Assisted Reproduction and Genetics, 30*(8), 1017–1022.
Yuan, J., Cha, J., Deng, W., Bartos, A., Sun, X., Ho, H. H., Borg, J. P., Yamaguchi, T. P., Yang, Y., & Dey, S. K. (2016). Planar cell polarity signaling in the uterus directs appropriate positioning of the crypt for embryo implantation. *Proceedings of the National Academy of Sciences of the United States of America, 113*(50), E8079–E8088.
Zhang, S., Kong, S., Wang, B., Cheng, X., Chen, Y., Wu, W., Wang, Q., Shi, J., Zhang, Y., Wang, S., Lu, J., Lydon, J. P., DeMayo, F., Pear, W. S., Han, H., Lin, H., Li, L., Wang, H., Wang, Y. L., Li, B., Chen, Q., Duan, E., & Wang, H. (2014). Uterine Rbpj is required for embryonic-uterine orientation and decidual remodeling via notch pathway-independent and -dependent mechanisms. *Cell Research, 24*(8), 925–942.

Uterine Receptivity: The Status of Uterus for Implantation

Jinhua Lu, Shuangbo Kong, and Haibin Wang, Xiamen University, Xiamen, Fujian, People's Republic of China; and Medical College of Xiamen University, Xiamen, Fujian, People's Republic of China

The Concept of Uterine Receptivity

In mammals, one of the most fascinating tissues is the uterus, whose major function is to accept implantation-competent blastocyst during a relatively short period defined as "the window of implantation." Correspondingly, the window of uterine receptivity is defined as the limited time when the uterus is suitable for embryo implantation. This concept was first raised and established by the studies employing the embryo transfer technique in the 1960s (Dickmann and Noyes, 1961). In rats, while preimplantation embryos on day 4 of pregnancy (day 1 = vaginal plug) showed severe damage 9 h after transfer into a day 5 uterus, blastocysts obtained from pregnant mice at day 5 can implant normally after transfer to either day 4 or day 5 uteri, but not in the uteri beyond day 5 of pregnancy or pseudopregnancy. These findings suggest that the uterus is not constantly receptive to blastocysts and embryo implantation could occur only in a limited period. This notion was further confirmed by the experiments conducted in mouse (Paria et al., 1993). On the basis of these previous findings, the uterine sensitivity to implantation-competent blastocysts is classically divided into three stages: prereceptive, receptive, and refractory phase. During the prereceptive stage, the uterus is favorable for embryo development but not suitable for implantation, while the uterus can initiate implantation when there are competent blastocysts at the receptive stage. During the refractory stage, however, implantation-competent blastocysts could not implant in the uterus, and the uterus is adverse to the survival and development of blastocyst (Wang and Dey, 2006). In mice, the uterus on days 1–3 of pregnancy is conventionally considered to be in the prereceptive phase. On day 4 of pregnancy, the uterus becomes fully receptive following the priming actions of ovarian progesterone and preimplantation estrogen, whereas the uterus fails to initiate implantation by late day 5. Similarly, the first 7 days of the secretory stage in the menstrual cycle is considered as the prereceptive stage in humans, 7–10 days after ovulation, is considered as the receptive stage, and the rest of the secretory stage is defined as the nonreceptive stage (Wang and Dey, 2006). It has been generally accepted that uterine receptivity is one of the pivotal events that determine the success of pregnancy, since implantation-competent blastocyst only implants in the uterus at the receptive state and any disturbance to uterine receptivity would lead to compromised pregnancy outcomes, including retarded embryo development and pregnancy failure.

The Uterine Preparation for Receptivity

The uterine tissue is composed of three major layers: the outer muscle layer, the inner luminal epithelium, and the stromal bed in between. Actually, changes in the uterus during pregnancy are hormone-dominant events, controlled by the steroid hormones from the ovaries. In essence, synchronization of the two major ovarian steroid hormones, estrogen and progesterone, directs the uterus into the receptive state, which exhibits the morphological and functional changes in the uterine epithelial and stromal cells (Wang and Dey, 2006).

Morphological Changes for Uterine Receptivity

Microvilli and pinopodes

The uterine lumen is lined by a polarized epithelium overlying the underlying stroma and myometrium. One of the morphological changes of the luminal epithelium that marks the uterine transition from prereceptive state to receptive state is the retraction of apical microvilli on the apical side of the luminal epithelial cells. These microvilli were the focus of many early ultrastructural studies and underwent dynamic changes in appearance in response to ovarian hormones. With progesterone alone, short regular microvilli are characteristically present, whereas estrogen alone results in long thin regular microvilli. Under the influence of either hormone alone, changes in the apical plasma membrane are mostly limited to alterations in the height and frequency of microvilli. However, when progesterone and estrogen act together leading to uterine receptivity for embryo implantation during the periimplantation period, the apical plasma membrane of uterine epithelial cells undergoes a more marked structural change during the several days of early pregnancy. More specifically, it gradually loses regular microvilli and becomes very flat. The apical plasma membrane of uterine epithelial cells from a microvillus to the flattened profile is a morphological sign of uterine receptivity in many species, since the uterus that lacks the apical membrane flattening is unable to support embryo implantation. Moreover, it has been established that regular microvilli begin to return to the apical plasma membrane very soon after the period of uterine receptivity, further indicating the close association between the membrane changes and uterine receptivity (Murphy, 2004).

Another morphological change of the luminal epithelium is the presence of large apical protrusions, marking the uterine transition from prereceptive state to receptive state. This structure was first discovered in rats and mice by traditional electron microscopy and was named as a "pinopode" because of its pinocytotic function (Nilsson, 1958). In rats, it has been shown that the development of pinopodes synchronizes with the window of uterine receptivity, since the number of pinopode increases on day 4 of pregnancy and becomes more abundant on day 5 when the uterus enters the receptive phase and decreases rapidly during the

postimplantation period. Pinopodes, the bulbous cytoplasmic protrusion projections on the apical surface of the luminal epithelium, appear only during the receptive phase. Therefore, these pinopodes are the best-studied ultrastructural markers of uterine receptivity, which are believed to be helpful in the attachment of the blastocyst to the surface of luminal epithelium. Further studies revealed that the dynamics of pinopodes in the uterus are under the control of ovarian steroid hormones. Actually, the appearance of pinopodes was found to be strictly progesterone-dependent. As to the estrogen, it is dose-dependent. While treatment with high doses of estradiol together with progesterone before the development of pinopodes inhibited pinopode formation, low doses of estradiol did not interfere with the process until the fourth day of treatment. Moreover, when estradiol was given as a single injection after pinopode formation, both doses of estrogen were equivalent in inducing the regression of pinopodes 48–72 h later (Martel et al., 1991). These findings demonstrate the similar hormonal conditions for both pinopode formation and the attainment of uterine receptivity, further confirming that the appearance of pinopodes is a well-defined histological marker for uterine receptivity. However, it is still questionable about whether human pinopodes are clinically useful to delineate the period of endometrial receptivity, since pinopodes are present throughout the luteal phase of the menstrual cycle. However, there are evidences that pinopodes are most prominent during the putative implantation window, suggesting that the pinopodes in human endometrium are at least helpful to the determination of uterine receptivity.

Luminal closure

Luminal closure, defined as the closure of uterine lumen during embryo apposition prior to attachment, is another morphological landmark for uterine receptivity. This event supports a closer contact between the luminal epithelium and the blastocysts, which is essential for appropriate blastocyst apposition and subsequent attachment. In rodents, a generalized stromal edema under the influence of ovarian steroid hormones leads to the closure of uterine luminal epithelium (Wang and Dey, 2006). And progesterone has been demonstrated to be essential for the luminal closure, since uterine luminal closure failed to occur in mice missing FK506-binding protein 4 (FKBP52), a cochaperone for full functions of progesterone receptor (PR) in the uterus. However, the occurrence of luminal closure does not require the presence of blastocysts, since this phenomenon could also be observed in both the pregnant and pseudopregnant uteri (Wang and Dey, 2006). Moreover, it has been shown that the secretion and reabsorption of uterine fluid is important for the uterine luminal closure and these processes are at least under the control of two major gatekeepers: cystic fibrosis transmembrane conductance regulator and epithelial Na+ channel (Tu et al., 2014).

Functional Changes for Uterine Receptivity

During the establishment of uterine receptivity, functional changes are mediated by several factors such as adhesion molecules, cytokines, and homeotic proteins. Many of these molecules have been identified as potential markers of uterine receptivity. For example, the glycoproteins expressed in the luminal epithelium are thought to function as a uterine barrier that inhibits the interaction between the blastocyst and luminal epithelium at the time of attachment. Unmasking of these glycoproteins at the implantation site correlates with increased blastocyst adhesion to the uterus (Dey et al., 2004). MUC1, a mucin-type glycoprotein, is integrally located in the apical plasma membrane of the luminal epithelium before implantation, whereas its expression is timely downregulated during the receptive period, in agreement with the view that glycoproteins act as uterine barrier that inhibits implantation. In humans, on the other hand, the expression of MUC1 remains at high levels during the implantation window, which seems to be contradictory to the antiadhesion function of MUC1. One explanation is that the embryo utilizes MUC1-associated glycans, which has been demonstrated in rabbit implantation. Actually, in vitro experiment using human blastocyst and endometrial epithelial cells indicates that the embryo could induce paracrine degeneration of epithelial-expressed MUC1 at the implantation site. Thus, it appears that MUC1 must be locally removed at the implantation site prior to successful blastocyst attachment in both human and animal models.

The Embryonic Contribution to Uterine Receptivity

Embryo implantation involves the implantation-competent embryo and the uterus at the receptive state. In addition to uterus itself, the embryo also plays an important role in determining the implantation window. Although it is clear that the states of the blastocyst determined the implantation window (Paria et al., 1993), there is a long-standing quest for specific embryo-derived molecules and signaling pathways that can functionally influence the states of the uterus, especially the receptive state of the uterus. One such important molecule is heparin-binding EGF-like growth factor (HB-EGF), encoded by Hbegf gene. Global gene analysis of the blastocysts with differential competency for implantation revealed that HB-EGF is significantly upregulated during blastocyst activation (Hamatani et al., 2004), suggesting that this molecule might be important for embryo implantation. This was confirmed by the experiments employing the blastocyst-size Affi-gel beads presoaked with HB-EGF protein. When these beads were transferred intraluminally into a pseudopregnant uterus, they can induce the expression of HB-EGF in uterine cells surrounding the beads and increase vascular permeability, similar to the physiological changes induced by normal blastocysts (Hamatani et al., 2004). Interestingly, previous studies demonstrated that HB-EGF is also expressed in the luminal epithelium at the site of blastocyst apposition approximately 6 h prior to blastocyst implantation in mice, with increasing expression of its receptors ErbB1 and ErbB4 and ligand-receptor binding activities observed in the blastocysts that are competent for implantation. Signaling by HB-EGF back to the embryo, in turn, activates blastocyst differentiation required for embryo adhesion during subsequent attachment and invasion. These observations

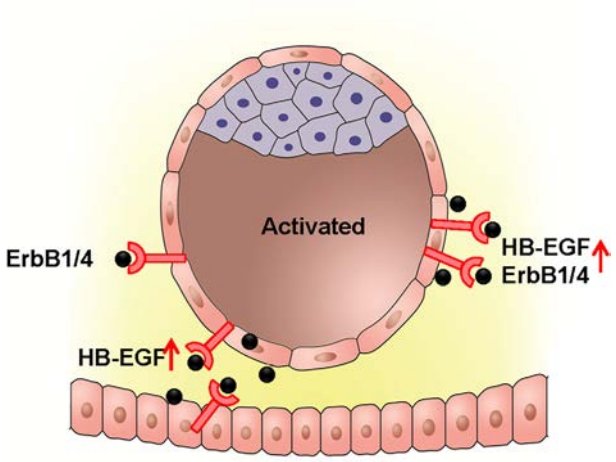


Fig. 1 The autoinduction loop of HB-EGF/ErbB4 between the implantation-competent blastocyst and the receptive uterus.

also suggest that HB-EGF conducts an autoinduction loop between the implanting blastocyst and the uterus via paracrine and juxtacrine signaling (Fig. 1). In human, HB-EGF is highly expressed in the receptive endometrium, and its receptor ErbB4 is localized on the surface of the trophectoderm in periimplantation blastocysts (Chobotova et al., 2002), indicating that HB-EGF/ErbB4 signaling also mediates the trophectoderm–uterine epithelium interaction during implantation in humans.

The Hormonal Control of Uterine Receptivity

Pregnancy is a hormone-dominant process, including the establishment of uterine receptivity, a key event and stage during normal pregnancy. The hormones that direct the acquisition of uterine receptivity are mainly the ovarian steroids estrogen and progesterone, although the hormonal requirements for uterine receptivity are species-dependent. While Progesterone is essential for the establishment of uterine receptivity in nearly all the mammals studied so far, the requirements for ovarian estrogen are species-specific. For instance, estrogen is essential for the acquisition of uterine receptivity in the rat and mouse, while maternal estrogen is not required for implantation in some species such as rabbit, hamster, pig, and guinea pig, with the explanation that blastocysts in these species have the ability to synthesize estrogen, which may contribute to the activation of implantation process. In other species, such as nonhuman primates and the human, the functions of estrogen during implantation remain inconclusive (Wang and Dey, 2006).

Estrogen is essential for uterine receptivity in the progesterone-primed uterus in mice. On day 1 of pregnancy in mice, under the influence of preovulatory ovarian estrogen, the uterine epithelial cells undergo extensive proliferation that to some extent continue through day 2. Rising progesterone levels secreted from the newly formed corpora luteum initiate the proliferation of uterine stromal cells from day 3 onward. On the morning of day 4, when the uterus is at the prereceptive stage, the production of a small amount of estrogen is crucial for the uterus to attain receptivity. At this time, the uterine epithelial cells gradually lose their polarity, and the apical plasma membranes of the epithelial cells become smooth and flattened at the site of blastocyst apposition. Ovariectomy immediately before the preimplantation estrogen secretion on day 4 and daily supplementation of progesterone beginning on day 5 result in blastocyst dormancy and inhibition of implantation, whereas a single injection of physiological levels of 17β-estradiol can induce the uteri from the neutral phase into the receptive state and renders the reactivation of blastocyst implantation (Zhang et al., 2013b). Based on these hormone profiles during the preimplantation period, exogenous estrogen and progesterone can also confer a receptive-stage uterus in ovariectomized mice.

In the uteri, estrogen and progesterone function mainly through nuclear estrogen receptors (ER) and PRs, respectively. Both the receptors have two main isoforms, known as ERα and ERβ for estrogen and PRA and PRB for progesterone. Pharmacological and genetic evidences have revealed the requirements of ER and PR for the preparation of uterine receptivity. For example, administration of antagonists for either ER or PR before implantation efficiently abolished the establishment of uterine receptivity. Moreover, previous works using knockout mice for ER or PR have also demonstrated their differential functions during uterine physiology. The uterus with Esr1 (gene encodes ERα) deletion is hypoplastic and unable to support implantation, whereas the uterus deficient for Esr2 (gene encodes ERβ) retains biological functions that allow for normal implantation, indicating the essential role of ERα during implantation. As to the PR, while mice with Pgr (gene encodes both the PRA and PRB with different promoters) deletion are infertile with defective ovarian and uterine functions, PRB-deficient females are fertile with normal ovarian and uterine responses, indicating that the functions of progesterone in uteri are primarily mediated by PRA (Zhang et al., 2013a).

The Establishment of Uterine Receptivity: Cell–Cell Interactions

Embryo implantation is a dynamic developmental event that involves a series of physical and physiological interactions among the blastocyst trophectoderm and various endometrial cell types. Similarly, the establishment of uterine receptivity also involves

multiple cell–cell interactions between the luminal and glandular epithelium and the stromal cells, which are under the primary influence of ovarian steroids estrogen and progesterone (Zhang et al., 2013b).

The Uterine Epithelial–Stromal Interaction

As previously mentioned, synchronization of estrogen and progesterone directs the uterus into a receptive state that is accompanied by obvious morphological and functional changes in the epithelium (Zhang et al., 2013a). The interactions of ovarian progesterone and estrogen during uterine cell proliferation and differentiation are summarized in Fig. 2 and are discussed later.

Estrogen binds stromal ERα to stimulate the proliferation of uterine epithelium via paracrine factors

ER is expressed in both epithelial and stromal cells of the adult uteri. It was initially assumed that estrogen acts directly through the ER in the corresponding compartments. However, evidences from the experiments employing Esr1 knockout mouse models and stromal–epithelial separation/recombination systems (Cunha, 2008) demonstrated that estrogen could not stimulate epithelial proliferation in genetically recombined uterine tissue that lacks stromal ERα, even in the presence of epithelial ERα. The tissue-specific knockout techniques render the selective deletion of Esr1 in the uterine epithelium and further proved that stromal ERα is responsible for estrogen-induced epithelial proliferation. As to how the estrogen–ERα activity in the stroma induces the epithelial proliferation, paracrine actions of polypeptide growth factors are believed to be essential for the uterine response to estrogens, such as insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF), or transforming growth factor α. Specifically, IGF-1 is an important growth factor that could be induced and activated immediately in the uterine stroma upon treatment with estrogen. It is necessary for estrogen-induced uterine epithelial proliferation through IGF-1 receptor in the luminal epithelium. Moreover, stromal ERα is also sufficient for estrogen-induced downregulation of PR in the uterine epithelial cells.

Differentiation of uterine epithelial cells requires both epithelial and stromal ERα

Although it is the uterine stromal ERα not the epithelial ERα that is indispensable for estrogen-induced epithelial proliferation, the epithelial ERα is also essential for complete biological functions in the uteri, since selective deletion of uterine epithelial Esr1 resulted in compromised increase of uterine weight induced by estrogen and led to epithelial apoptosis after initial proliferation. Further evidence revealed that both the stromal and epithelial ERα are required for the functional differentiation of uterine epithelium, which could be indicated by such secretory proteins as lactoferrin, complement component C3, and MUC1 (Buchanan et al., 1999).

Progesterone functions through stromal PR to antagonize the epithelial proliferative response to estrogen and induce stromal proliferation

The phenotypes of uteri with Pgr-null mutation are similar to that of the uteri in the ovariectomized mice exposed to prolonged estrogen treatment. This finding revealed an essential role of PR in the uterus. Furthermore, recombination experiments using uterine tissue from Pgr-null mice demonstrated that stromal PR is required for progesterone to decrease the proliferation-promoting effect of estrogen on the uterine epithelial cells and promote the proliferation of stromal cells. And numerous molecules have been identified to mediate progesterone–PR signaling, such as immunophilin FK506-binding protein 4 (FKbp52), chicken ovalbumin upstream promoter-transcription factor II, the basic helix–loop–helix transcription factor, and heart and neural crest derivatives expressed transcript 2 (Hand2).

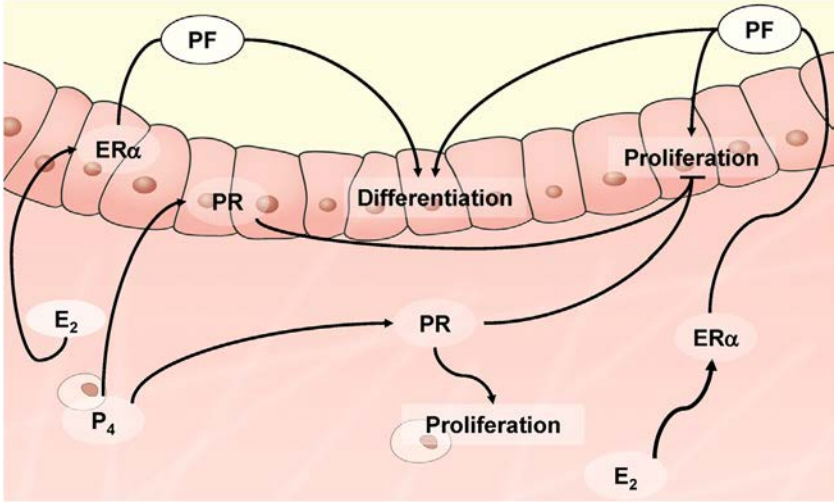


Fig. 2 The uterine epithelial–stromal interactions mediated by the ovarian progesterone and estrogen and their respective receptors.

Epithelial PR is also involved in stromal–epithelial cross talk

Despite the well-established concept that stromal PR mediates the antagonistic functions of progesterone on the epithelial proliferative response to estrogen, the important roles of epithelial PR in uterine biology were largely ignored. A recent study with Pgr deficiency in the uterine epithelial cells has demonstrated that epithelial PR is essential for epithelial expression of Indian hedgehog, which participates in the uterine stromal–epithelial cross talk. The absence of epithelial Pgr results in complete failure of pregnancy due to impaired uterine receptivity. This finding clearly demonstrated that epithelium PR is indispensable for the stromal–epithelial interactions during the establishment of normal uterine receptivity.

The Uterine Glandular–Epithelial Interaction

All mammalian uteri contain glands in the endometrium that synthesize and secrete substances essential for the survival and development of the conceptus. It has been shown that uterine glands and their paracrine-acting secretions have important biological roles in the establishment of uterine receptivity and blastocyst implantation (Kelleher et al., 2016). Of the substances secreted by uterine glands, LIF is one of the most important molecules, since Lif null mutation in mice led to infertility, defective uterine receptivity, and failed blastocyst implantation. The LIF is secreted into the uterine lumen where it binds the LIF-receptor complex composed of LIF receptor (LIFR) and gp130. The overlapping expression pattern of LIFR and gp130 in the luminal epithelium provides robust evidence for the paracrine nature of glandular LIF acting on uterine epithelial cells. Failure of gp130 activation in the luminal epithelium in Lif null mice reinforces the notion that LIF-driven glandular–luminal epithelial interaction is essential for normal uterine receptivity and blastocyst implantation. Although LIF has a well-recognized role to establish receptivity of the luminal epithelium for implantation, the role of many other genes expressed in the uterine glands has not been established. Moreover, future studies are needed to determine the role of the uterine glands and their secretions in paracrine interactions with the luminal epithelial cells.

The Flexibility of Uterine Receptivity

Since the uterine receptive is determined by the ovarian steroid hormones estrogen and progesterone, the states of the uterine receptive could be changed by the manipulation of the hormones estrogen and progesterone (Zhang et al., 2013a).

Estrogen Is Critical for Specifying the Duration of Uterine Receptivity

In rodents, estrogen is essential for the preparation of a progesterone-primed uterus to the receptive state. Ovariectomy conducted before preimplantation estrogen secretion on the morning of day 4 results in blastocyst dormancy and inhibition of implantation, also known as delayed implantation. This neutral uterine phase can be maintained by continued progesterone treatment but is terminated by estrogen injection. The impacts of different doses of estrogens on the length of implantation window have been explored using this delayed implantation model. For instance, estrogen at a low threshold level extends the window of uterine receptivity, whereas physiological higher levels of estrogen rapidly shut off the implantation window, transforming the uterus into a refractory state. The views that high levels of estrogen are detrimental to implantation are further supported by the findings that ovarian hyperstimulation leads to implantation failure and embryo resorption. In humans, the life span of fully developed pinopodes lasts maximally 48 h, suggesting a transient cell state in the receptive endometrium. Following ovarian stimulation with clomiphene citrate and human chorionic gonadotropin, pinopodes formed 1–2 days earlier than that in the natural cycles. Early pinopode formation caused by ovarian stimulation may suggest the shifting of the window for receptivity. Based on these findings, it is reasonable to speculate that reduced implantation in the cycles of in vitro fertilization could be due to asynchrony between the receptive endometrium and implantation-competent blastocyst that result from exposure to excessive estrogen.

Progesterone Supplementation Extends the Window of Uterine Receptivity

In mice, blastocysts can initiate implantation out of the normal "window" of uterine receptivity. For example, blastocysts can still initiate implantation when transferred on day 5 of pseudopregnancy, but implantation will not occur when normal blastocysts are transferred into day 6 pseudopregnant uteri. Exogenous progesterone supplementation can prolong the implantation window to day 6. However, deferred embryo implantation beyond the normal "window" of uterine receptivity leads to embryonic demise before birth in mice and is often associated with higher risk of early pregnancy losses in humans (Wang and Dey, 2006).

References

Buchanan, D. L., Setiawan, T., Lubahn, D. B., Taylor, J. A., Kurita, T., Cunha, G. R., & Cooke, P. S. (1999). Tissue compartment-specific estrogen receptor-alpha participation in the mouse uterine epithelial secretory response. *Endocrinology, 140*, 484–491.

Chobotova, K., Spyropoulou, I., Carver, J., Manek, S., Heath, J. K., Gullick, W. J., Barlow, D. H., Sargent, I. L., & Mardon, H. J. (2002). Heparin-binding epidermal growth factor and its receptor ErbB4 mediate implantation of the human blastocyst. *Mechanisms of Development, 119*, 137–144.

Cunha, G. R. (2008). Mesenchymal-epithelial interactions: past, present, and future. *Differentiation, 76*, 578–586.
Dey, S. K., Lim, H., Das, S. K., Reese, J., Paria, B. C., Daikoku, T., & Wang, H. (2004). Molecular cues to implantation. *Endocrine Reviews, 25*, 341–373.
Dickmann, Z., & Noyes, R. W. (1961). The zona pellucida at the time of implantation. *Fertility and Sterility, 12*, 310–318.
Hamatani, T., Daikoku, T., Wang, H., Matsumoto, H., Carter, M. G., Ko, M. S., & Dey, S. K. (2004). Global gene expression analysis identifies molecular pathways distinguishing blastocyst dormancy and activation. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 10326–10331.
Kelleher, A. M., Burns, G. W., Behura, S., Wu, G., & Spencer, T. E. (2016). Uterine glands impact uterine receptivity, luminal fluid homeostasis and blastocyst implantation. *Scientific Reports, 6*, 38078.
Martel, D., Monier, M. N., Roche, D., & Psychoyos, A. (1991). Hormonal dependence of pinopode formation at the uterine luminal surface. *Human Reproduction, 6*, 597–603.
Murphy, C. R. (2004). Uterine receptivity and the plasma membrane transformation. *Cell Research, 14*, 259–267.
Nilsson, O. (1958). Ultrastructure of mouse uterine surface epithelium under different estrogenic influences. 2. Early effect of estrogen administered to spayed animals. *Journal of Ultrastructure Research, 2*, 73–95.
Paria, B. C., Huet-Hudson, Y. M., & Dey, S. K. (1993). Blastocyst's state of activity determines the "window" of implantation in the receptive mouse uterus. *Proceedings of the National Academy of Sciences of the United States of America, 90*, 10159–10162.
Tu, Z., Ran, H., Zhang, S., Xia, G., Wang, B., & Wang, H. (2014). Molecular determinants of uterine receptivity. *International Journal of Developmental Biology, 58*, 147–154.
Wang, H., & Dey, S. K. (2006). Roadmap to embryo implantation: clues from mouse models. *Nature Reviews. Genetics, 7*, 185–199.
Zhang, S., Kong, S., Lu, J., Wang, Q., Chen, Y., Wang, W., Wang, B., & Wang, H. (2013a). Deciphering the molecular basis of uterine receptivity. *Molecular Reproduction and Development, 80*, 8–21.
Zhang, S., Lin, H., Kong, S., Wang, S., Wang, H., Wang, H., & Armant, D. R. (2013b). Physiological and molecular determinants of embryo implantation. *Molecular Aspects of Medicine, 34*, 939–980.

"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 – Fertilization & Implantation Systems
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
April 9, 2024
Week 14

Fertilization & Implantation Systems

Fertilization –
- Sperm and female reproductive tract
- Attraction, hyperactivation, binding, acrosome reaction
- Penetration, sperm-egg fusion
- PLC and calcium mobilization
- Fertilization and embryo induction

Implantation –
- Embryo development and fallopian tube
- Endocrine induction of uterine development
- Uterine cell biology, vascularization and maturation
- Proliferative and secretory stage
- Blastula and endometrium interactions
- Implantation apposition, adhesion, invasion and system biology

Required Reading

Evans JP. (2018) Fertilization in the Oviduct. In: Encyclopedia of Reproduction 2nd Edition, Ed: MK Skinner. Elsevier. Vol 3:300-304.
Deguchi R, Hirohashi N. (2018) Fertilization, Comparative. In: Encyclopedia of Reproduction 2nd Edition, Ed: MK Skinner. Elsevier. Vol 6:344-349.
Cha JM, et al. (2018) Aspects of Rodent Implantation. In: Encyclopedia of Reproduction 2nd Edition, Ed: MK Skinner. Elsevier. Vol 2:291-297.
Lu J, Kong S, Wang H. (2018) Uterine Receptivity: The Status of Uterus for Implantation. In: Encyclopedia of Reproduction 2nd Edition, Ed: MK Skinner. Elsevier. Vol 2:394-399.

Spring 2024 – Systems Biology of Reproduction
Discussion Outline – Fertilization & Implantation Systems
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
April 11, 2024
Week 14

Fertilization & Implantation Systems

Primary Papers:

1. Teperek, et al. (2016) Genome Research 26:1034.
2. Stenhouse, et al. (2022) Recent Advances in Animal Nutrition and Metabolism, Adv Exp Med Biol 1354 :25-48.
3. Vento-Tormo, et al. (2018) Nature 563(7731):347-353.

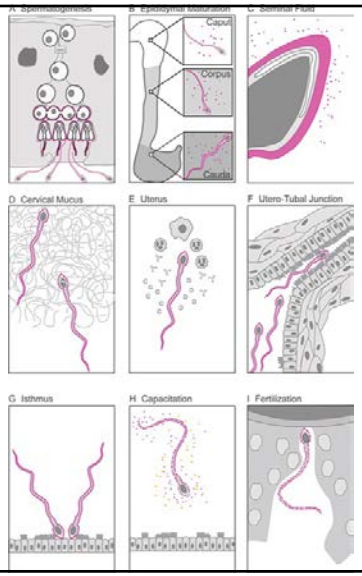
Discussion

Student 1: Reference 1 above
- What was the experimental design and objectives?
- What impact on the developing embryo was observed?
- Can sperm epigenetic alterations modify the embryo?

Student 2: Reference 2 above
- How do embryos interact with endometrial cells?
- What factors were observed?
- How does implantation and placentation vary between humans, rodents, sheep and pigs?

Student 3: Reference 3 above
- What was the experimental design and technology?
- What maternal-fetal interface interactions were identified?
- What new insights for maternal-fetal interface were found to be critical for placentation and reproductive success?

Fertilization



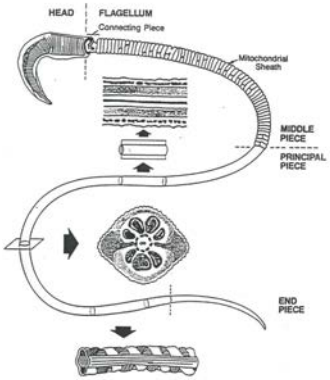


FIG. 1. General features of the mammalian sperm. The head of the spermatozoon is attached to the connecting piece of the flagellum. The other regions of the flagellum are the middle piece, the principal piece, and the end piece. The middle piece contains the mitochondrial sheath, while the principal piece contains the fibrous sheath. Longitudinal and cross-sectional views of the principal piece and a segment of fibrous sheath are indicated by arrows; the internal components of the flagellum are identified in Fig. 9.

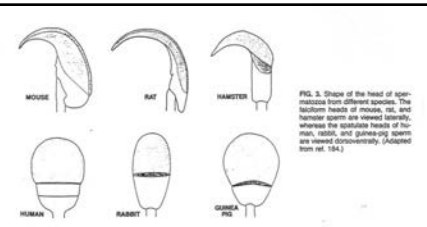


FIG. 3. Shape of the head of spermatozoa from different species. The falciform heads of mouse, rat, and hamster sperm are viewed laterally, whereas the spatulate heads of human, rabbit, and guinea-pig sperm are viewed dorsoventrally. (Adapted from ref. 184.)

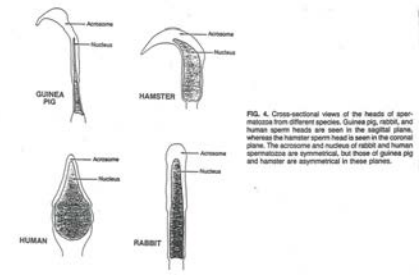


FIG. 4. Cross-sectional views of the heads of spermatozoa from different species. Guinea pig, rabbit, and human sperm heads are seen in the sagittal plane, whereas the hamster sperm head is seen in the coronal plane. The acrosome and nucleus of rabbit and human spermatozoa are symmetrical, but those of guinea pig and hamster are asymmetrical in these planes.

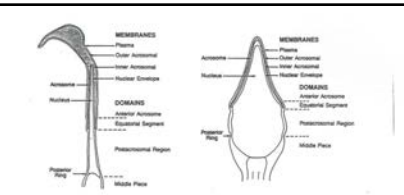


FIG. 6. Structure of the head of guinea-pig and human spermatozoa. The plasma membrane in the anterior acrosomal and equatorial segment domains overlies the outer acrosomal membrane. The inner acrosomal membrane, in turn, overlies the nuclear envelope. The acrosome is thinner in the equatorial segment region than in the anterior acrosome region. The posterior ring separates the postacrosomal domain of the head from the middle-piece domain of the flagellum.

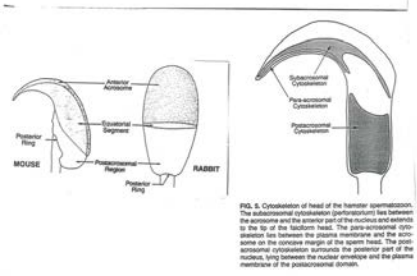


FIG. 5. Cytoskeleton of head of the hamster spermatozoon. The subacrosomal cytoskeleton (perforatorium) lies between the acrosome and the anterior part of the nucleus and extends to the tip of the falciform head. The para-acrosomal cytoskeleton lies between the plasma membrane and the acrosome on the concave margin of the sperm head. The postacrosomal cytoskeleton surrounds the posterior part of the nucleus, lying between the nuclear envelope and the plasma membrane of the postacrosomal domain.

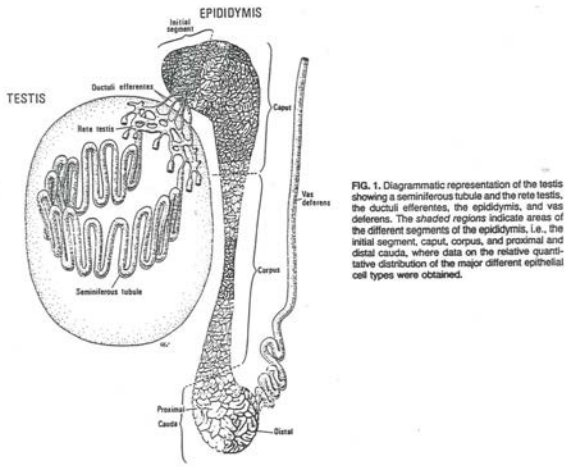


FIG. 1. Diagrammatic representation of the testis showing a seminiferous tubule and the rete testis, the ductuli efferentes, the epididymis, and vas deferens. The *shaded regions* indicate areas of the different segments of the epididymis, i.e., the initial segment, caput, corpus, and proximal and distal cauda, where data on the relative quantitative distribution of the major different epithelial cell types were obtained.

FIGURE 3. Changes in the beating pattern of golden hamster spermatozoa during the development of sperm motility: a, testicular; b, caput epididymal; and c, cauda epididymal spermatozoa. The time interval between successive images is 1.5 sec in a, 0.5 sec in b, and 1/60 sec in c. The scale bar = 30 μm.

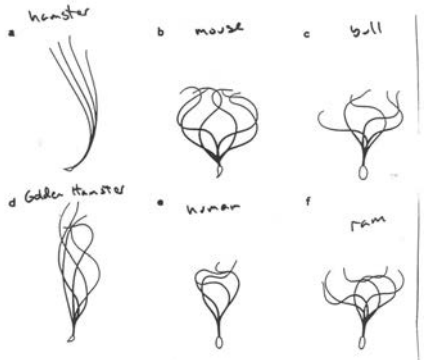
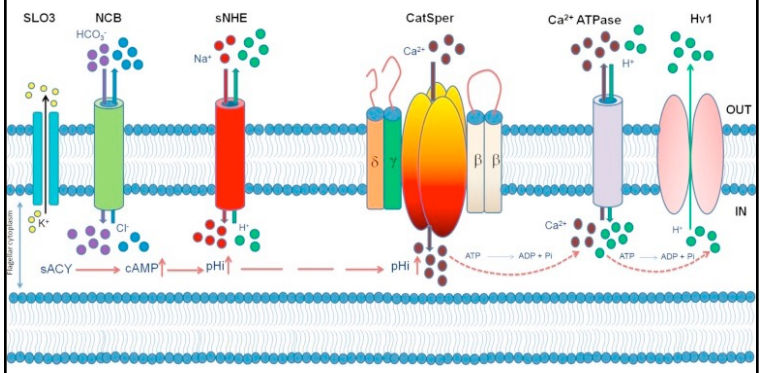
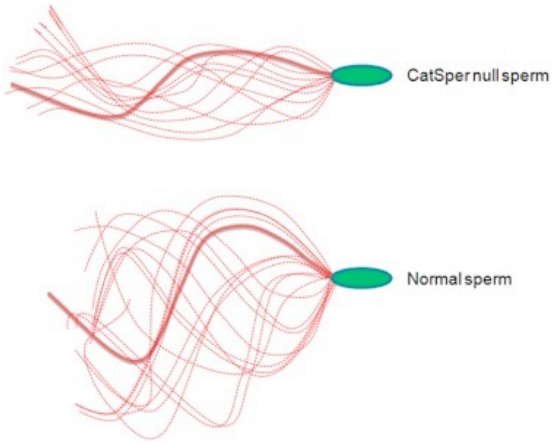


FIGURE 1. Tracings of flagellar waveform of intact mammalian spermatozoa. Each figure traced from published micrographs has been superimposed so that the head and the proximal part of the midpiece of each tracing is exactly aligned in order to easily distinguish the differences in the waveforms: a, Chinese hamster; b, mouse; c, bull; d, golden hamster; e, human; f, ram. The time interval between successive images is 1/150 sec in a, 1/60 sec in b, d, e, and f, and 1/66 sec in c. The scale bar = 30 μm in both d (for a and d) and f (for b, c, e, and f). (a) Adapted from Phillips, D. M., *J. Cell Biol.*, 53, 561, 1972. With permission. (b) Redrawn from Wolf, D. E., Hagopian, S. S., and Ishijima, S., *J. Cell Biol.*, 102, 1372, 1986. With permission. (c) Adapted from Gray, J., *J. Exp. Biol.*, 35, 96, 1958. With permission. (d) Modified from Ishijima, S. and Mohri, H., *J. Exp. Biol.*, 114, 463, 1985. With permission. (e) Modified from Ishijima, S., Oshio, S., and Mohri, H., *Gamete Res.*, 13, 185, 1986. With permission. (f) Redrawn from Ishijima, S. and Witman, G. B., *Cell Motil. Cytoskeleton*, 8, 375, 1987. With permission.

CatSper channel, sperm function and male fertility.
Singh AP, Rajender S.
Reprod Biomed Online. 2015 Jan;30(1):28-38.



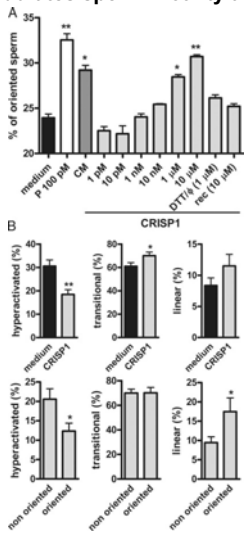


Hypermotility: normal sperm display vigorous and asymmetrical flagellar beating termed as hypermotility, which is essential for propagation through viscous ovi-ductal fluid. CatSper null sperm show only basal motility and are unable to propagate to destination and penetrate protective layers of the egg.

CRISP1 as a novel CatSper regulator that modulates sperm motility and orientation during fertilization.

Ernesto JI, Weigel Muñoz M, Battistone MA, et al.
J Cell Biol. 2015 Sep 28;210(7):1213-24.

Sperm orientation and motility in the presence of CRISP1. (A) Capacitated sperm were placed in one well of a modified Zigmond chamber and CRISP1 (1 pM to 10 µM), DTT-treated and heat-denatured CRISP1 (1 µM; DTT/Φ), or recombinant CRISP1 (10 µM; rec) were loaded in the second well. Medium alone was used as negative control and both progesterone (100 pM; P) and cumulus-conditioned medium (CM) were used as positive controls. After 15 min, the percentage of oriented sperm toward the corresponding gradients was calculated by analyzing sperm trajectories. In all cases, results represent the mean ± SEM of at least three independent experiments in which >150 sperm trajectories per experiment were analyzed. *, $P < 0.05$; **, $P < 0.005$ vs. medium. (B) Percentages of hyperactivated (left), transitional (middle), or linear (right) patterns of motility for sperm exposed to either CRISP1 (1 µM) or medium (control; top) and for oriented and nonoriented cells within the CRISP1-exposed group (bottom). In all cases, results represent the mean ± SEM of seven independent experiments in which at least 100 sperm trajectories per experiment were analyzed. **, $P < 0.005$; *, $P < 0.05$.

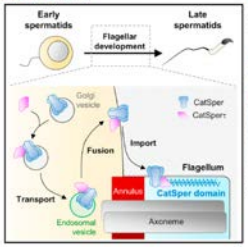


Article

Cell Reports

***C2cd6*-encoded CatSper$_\tau$ targets sperm calcium channel to Ca^{2+} signaling domains in the flagellar membrane**

Graphical abstract



Authors
Jae Yeon Hwang, Huafeng Wang, Yonggang Lu, Masahito Ikawa, Jean-Ju Chung

Correspondence
jean-ju.chung@yale.edu

In brief

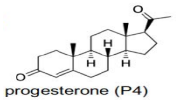
Hwang et al. report that the C2 domain protein CatSper$_\tau$ targets the sperm CatSper Ca^{2+} channel to linear domains of the sperm flagellum during development. The findings provide fundamental insights into CatSper trafficking and the shared molecular mechanisms among ciliary and flagellar membrane targeting.

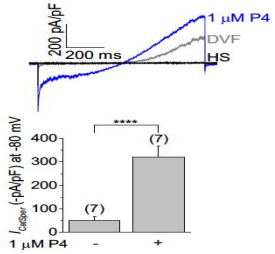
Highlights

- CatSper$_\tau$ encoded by C2cd6 is a C2 membrane-associating domain-containing protein
- CatSper$_\tau$ loss of function impairs sperm hyperactivation and male fertility
- CatSper$_\tau$ adopts ciliary trafficking machineries for flagellar targeting via C2 domain
- CatSper$_\tau$ targets the CatSper channel into nanodomains of developing sperm flagella

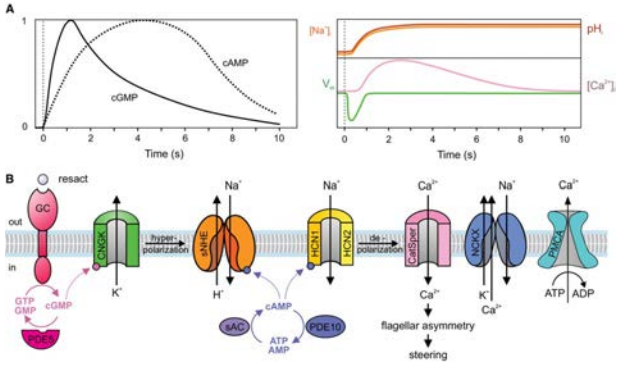
Hwang et al., 2022, Cell Reports 38, 110226
January 18, 2022 © 2021 The Authors.

Regulation of the sperm calcium channel CatSper by endogenous steroids and plant triterpenoids.
Proc Natl Acad Sci U S A. 2017 May 30;114(22):5743-5748.
Mannowetz N, Miller MR, Lishko PV.





Absolute proteomic quantification reveals design principles of sperm flagellar chemosensation.
Trötschel C, Hamzeh H, Alvarez L, et al.
EMBO J. 2020 Feb 17;39(4):e102723.

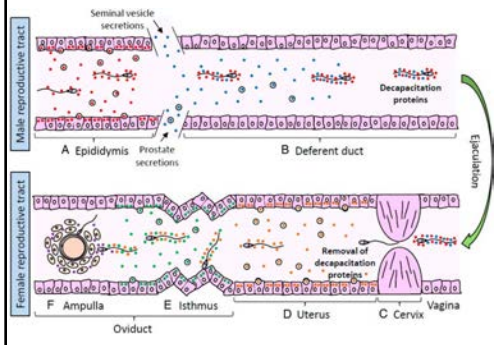


Ram seminal plasma and its functional proteomic assessment.
Leahy T, Rickard JP, Bernecic NC, Druart X, de Graaf SP.
Reproduction. 2019 Jun;157(6):R243-R256.

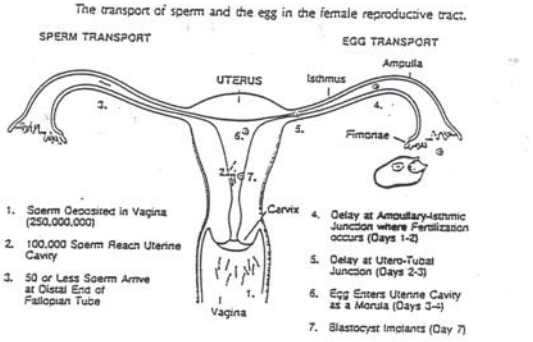
Table 1 The major proteins identified in ram seminal plasma after LC-MS/MS, based on total spectra counts (Soleilhavoup *et al.* 2014).

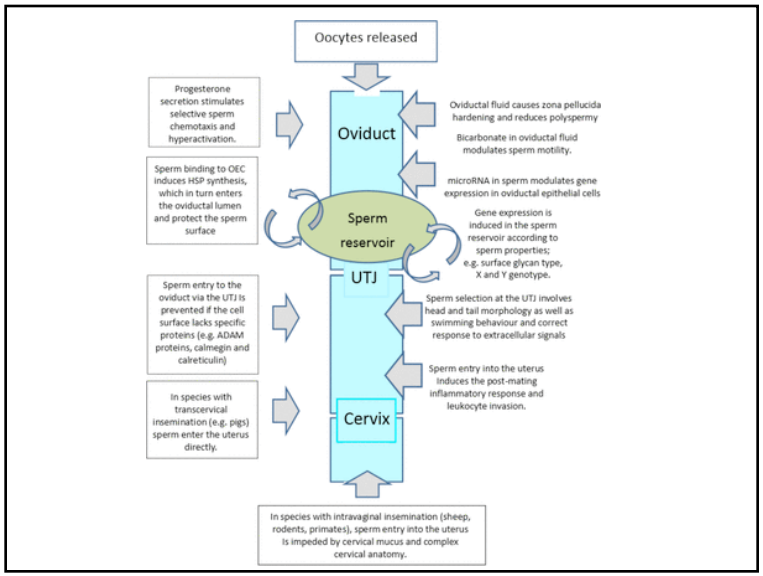
Protein name	Gene symbol	MW (kDa)	Function
Binder of sperm 5 precursor	*BSP5*	17.8	Binder of sperm (BSP) glycoprotein characterised by a fibronectin type-2 domain. Binds sperm
UPF0762 protein C6orf58	*LEG1*	40.9	Protein of unknown function
Clusterin	*CLU*	51.0	Ubiquitous glycoprotein with chaperone and anti-apoptotic functions
Bodhesin-2	*BDH2*	11.7	Spermadhesin protein characterised by a CUB domain. Binds sperm
Alpha-2-macroglobulin	*A2M*	164.2	Protease inhibitor
Carboxylesterase 5A	*CES5A*	64.2	Enzyme involved with lipid transfer processes
Lactoferrin	*LTF*	77.2	Antimicrobial activity and serine-type endopeptidase activity. Iron binding
EGF-like repeat and discoidin I-like domain-containing protein 3	*EDIL3*	54.9	Cell adhesion ligand that interacts with the alpha-v/beta-3 integrin receptor. Calcium binding

Proteins from male and female reproductive tracts involved in sperm function regulation.
Hernández-Silva G, Chirinos M.
Zygote. 2019 Feb;27(1):5-16.

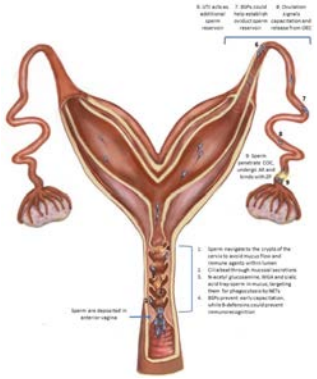


Sperm interaction with proteins from male and female reproductive tracts. (*A*) Spermatozoa released from the seminiferous tubules interact with epididymal proteins (red circles) that promote post-testicular maturation. (*B*) During ejaculation, spermatozoa are transported to the vas deferens and mixed up with seminal vesicle and prostate secretions, containing proteins that interact with the sperm surface and act as decapacitation factors (blue circles). (*C*) When semen is deposited in the female reproductive tract, decapacitation proteins are removed from spermatozoa during their passage through the cervical mucus in the cervix. (*D*) Spermatozoa migrating towards the uterus are exposed local secreted proteins (orange circles) that regulate the progress of sperm capacitation. (*E*) Once in the oviduct, spermatozoa may either move forward to the ampulla stimulated by secreted proteins (green circles) or held on the epithelium surface of mucosal folds in the isthmus to create a sperm reservoir. When ovulation draws near, local secretions stimulate the sperm release from the reservoir and the resumption of capacitation. (*F*) Spermatozoa that successfully reach the cumulus–oocyte complex are exposed to follicular fluid proteins (purple circles) which promote sperm penetration of the oocyte vestments and fertilization.

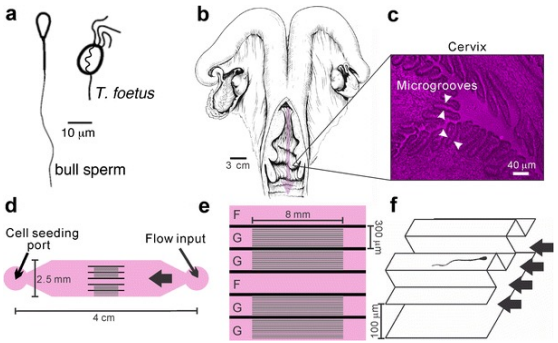




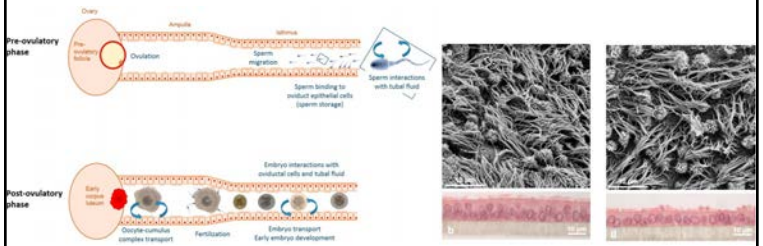
Sperm surface changes and their consequences for sperm transit through the female reproductive tract.
Rickard JP, de Graaf SP.
Theriogenology. 2020 Feb 10. [Epub ahead of print]



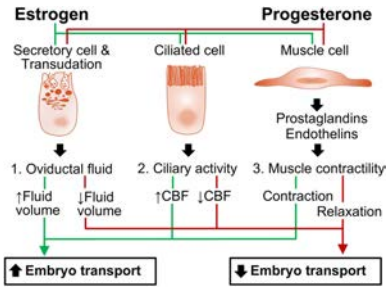
Mammalian sperm interactions with the female reproductive tract.
Suarez SS.
Cell Tissue Res. 2016 Jan;363(1):185-194.

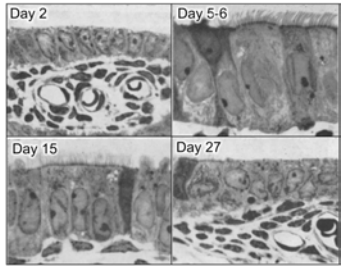


Composing the Early Embryonic Microenvironment: Physiology and Regulation of Oviductal Secretions.
Saint-Dizier M, Schoen J, Chen S, Banliat C, Mermillod P.
Int J Mol Sci. 2019 Dec 28;21(1).



Roles of steroid hormones in oviductal function
Barton BE, Herrera GG, Anamthathmakula P, Rock JK, Willie A, Harris EA, Takemaru KI, Winuthayanon W. Reproduction. 2020 Mar 1;159(3):R125-R137.





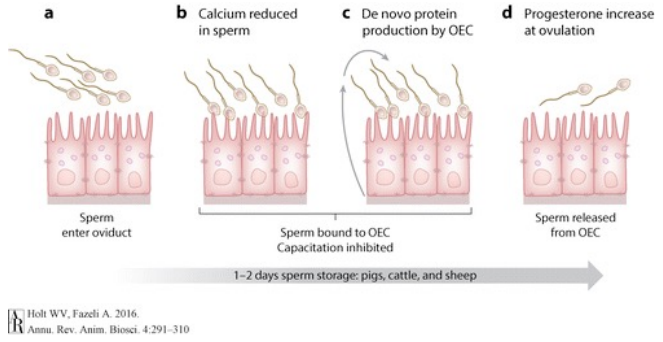
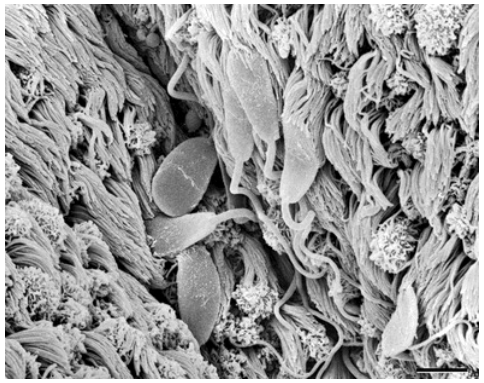


Diagram summarizing the main events that occur during oviductal sperm storage in pigs, cattle, and sheep. (a) Spermatozoa enter the oviductal isthmus via the utero-tubal junction. (b) Some spermatozoa bind to oviductal epithelial cell (OEC) surfaces, whereupon their motility and intracellular calcium concentrations are reduced; capacitation is also inhibited. (c) The direct contact between spermatozoa and epithelial cells induces de novo gene expression and protein synthesis. Multiple proteins, including heat shock proteins, are secreted into the oviductal lumen, where they protect sperm membranes and facilitate sperm storage. (d) Increased peri-ovulatory progesterone production induces spermatozoa to escape and resume their progress toward the oocyte(s).



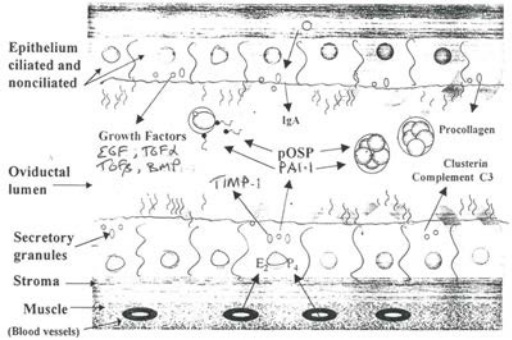
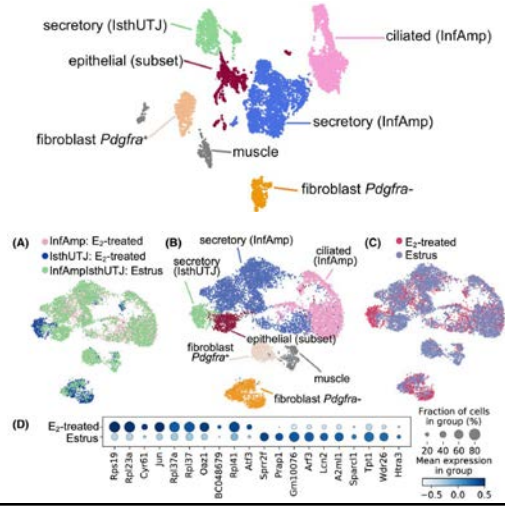
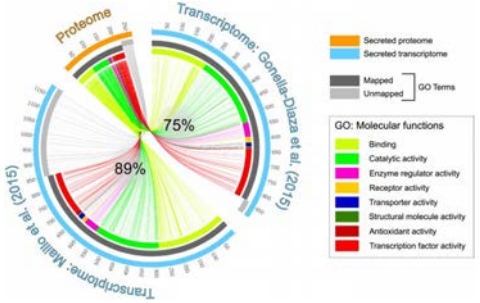


Fig. 9. Ciliated and secretory epithelium achieve maximum development, biosynthetic capacity and secretory activity during follicular development, ovulation and early cleavage-stage development. Secretory products are contributed as transudate and from active biosynthesis by secretory epithelium into the oviductal microenvironment. These molecules may operate in an autocrine and/or paracrine manner to regulate oviductal and embryonic growth and development.

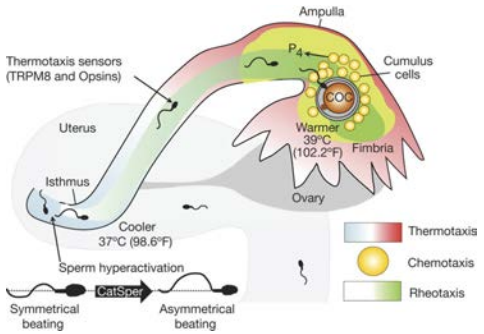
Cell-type specific analysis of physiological action of estrogen in mouse oviducts.
McGlade EA, Herrera GG, Stephens KK, et al.
FASEB J. 2021 May;35(5):e21563.



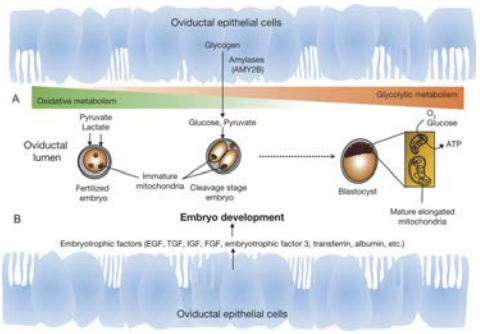
Profiling of proteins secreted in the bovine oviduct reveals diverse functions of this luminal microenvironment.
PLoS One. 2017, 12(11):e0188105.
Pillai VV, Weber DM, Phinney BS, Selvaraj V.



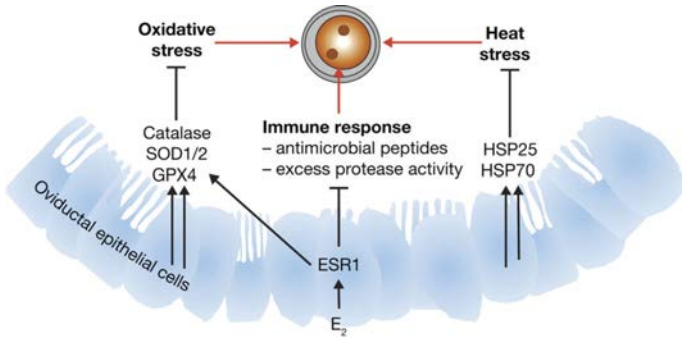
Comparison of oviductal cell secreted proteins to transcriptome of the bovine oviduct from two published datasets. Of the 266 secreted proteins identified in oviductal cells, 236 (89%) and 200 (75%) of the proteins were detected as transcripts in the oviduct by Maillo et al. [35], and the Gonella-Diaza et al. [36] respectively. This comparison indicates that almost 90% of the proteins identified in this study are synthesized by the oviductal epithelium, with only 30 (11%) of proteins putatively derived by plasma protein transudation.



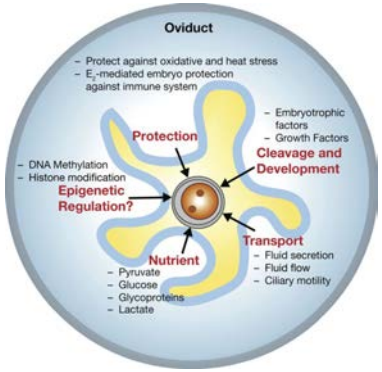
Oviduct-guided fertilization. The oviduct regulates fertilization through sperm guidance and sperm hyperactivation. The sperm guidance is achieved through rheotaxis, thermotaxis and chemotaxis. Rheotaxis is created by tubal fluid, which generates a current flow from the ampulla toward the isthmus of the oviduct. Sperm swim against this current based on the physical rotation of the flagella upon CatSper (Cation channel of Sperm) activation. Thermotaxis is mediated through a Ca^{2+}-sensing transient receptor potential channel (TRPM8) and G protein-coupled receptor (opsins). This thermal sensibility of human sperm can detect a difference of 0.006˚ C (Bahat et al. 2012). Temperatures depicted (37˚ C vs 39˚ C) are from the finding in rabbits (Bahat et al. 2003). Chemotaxis is driven through progesterone (P4) released from the cumulus cells and through small cytokines found in the follicular fluid. Together, these processes provide guidance for sperm to swim toward the eggs and be competent for fertilization. COC, cumulus-oocyte complex.



The influence of the oviduct on embryo development. (A) The main energy supply for the embryos is pyruvate and lactate. During the very early stage of embryo development, pyruvate and lactate are provided by the oviductal fluid as energy sources for oxidative metabolism. The oviduct can also supply the glycogen as an energy source for the embryos during the cleavage stage. Amylase (AMY2B) is produced within the oviductal epithelial cells and converts glycogen to sugar. At this stage, the mitochondria of the embryos are immature and do not function. During morula and blastocyst stages, the mitochondria are fully mature and can use oxygen and glucose to produce their own energy via glycolysis as they leave the oviduct. (B) At the same time, oviductal epithelial cells provide embryotrophic factors, such as growth factors, to promote cleavage and embryo development. EGF, epidermal growth factor; FGF, fibroblast growth factor; IGF, insulin-like growth factor; TGF, transforming growth factor.

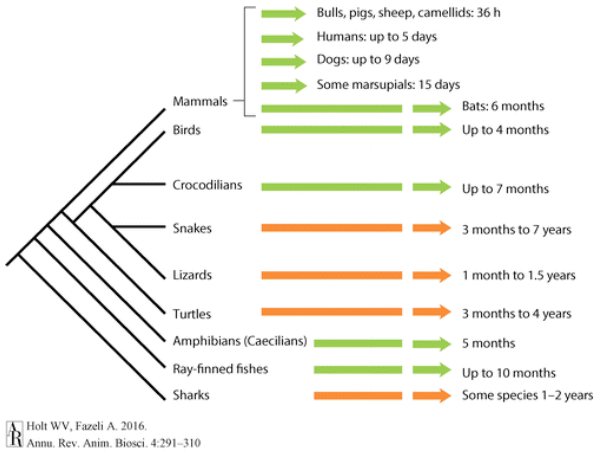


Oviduct-mediated embryo protection against stress and immune responses. Oviductal epithelial cells produce heat shock protein family (HSP25 and HSP70) to handle heat stress. Oviductal fluid also contains catalase, superoxide dismutases (SOD1/2) and glutathione peroxidase (GPX4) to reduce stress of the embryos from reactive oxygen species. In addition to stress, the oviduct also protects the embryos against their own immune system, partly through the E_2/ESR1 signaling, by inhibiting the production of antimicrobial peptides and excess protease activity. ESR1, estrogen receptor α.

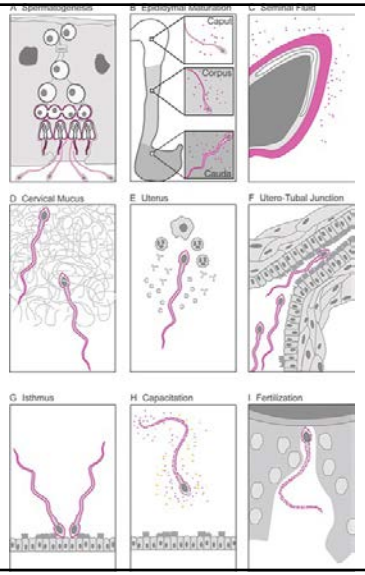


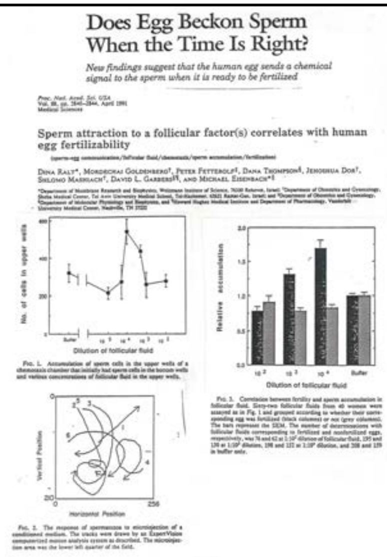
Essential roles of the oviduct in assisting embryo development. Schematic cross-section of the oviduct and the embryo with their interaction regulating embryo development. The interactions are divided into five domains: protection, cleavage and development, transport, nutrients and epigenetic regulation. E_2, estrogen.

Sperm Storage in the Female Reproductive Tract.
Holt WV, Fazeli A.
Annu Rev Anim Biosci. 2016 Feb 15;4:291-310.



Holt WV, Fazeli A. 2016.
Annu. Rev. Anim. Biosci. 4:291–310





Does Egg Beckon Sperm When the Time Is Right?

New findings suggest that the human egg sends a chemical signal to the sperm when it is ready to be fertilized

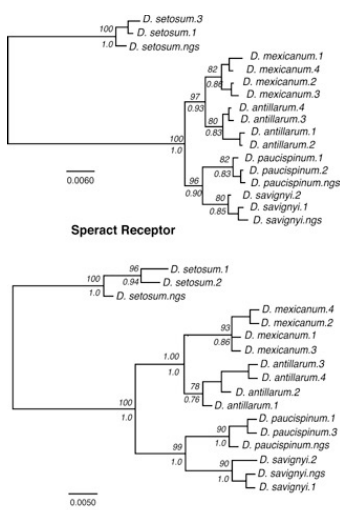
Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 2840–2844, April 1991
Medical Sciences

Sperm attraction to a follicular factor(s) correlates with human egg fertilizability

Dina Ralt, Mordechai Goldenberg, Peter Fetterolf, Dana Thompson, Jehoshua Dor, Shlomo Mashiach, David L. Garbers, and Michael Eisenbach

TABLE 1
SUMMARY OF CHARACTERIZED CHEMOKINETIC AND CHEMOTACTIC MOLECULES AND THEIR EFFECTS ON SPERM

Species	Name	Structure	Derivatives*	Actions
Strongylocentrous purpuratus *Hemicentrotus pulcherrimus* (sea urchin)	Speract (SAP I)[b,c,d]	Gly-Phe-Asp-Leu-Asn-Gly-Gly-Gly-Val-Gly	36[e,f,g,h]	↑cAMP, ↑cGMP ↑Ca_i^{2+}, ↑pH_i ↑Respiration ↑Motility Cofactor in AR (?)
Arbacia punctulata (sea urchin)	Resact (SAP II)[i]	Cys-Val-Thr-Gly-Ala-Pro-Gly-Cys-Val-Gly-Gly-Gly-Arg-Leu-NH_2	?	↑cAMP, ↑cGMP ↑Respiration ↑Motility ↑Guanylyl cyclase Chemoattractant
Glyptocidaris crenularis *Stomopneustes variolaris* (sea urchin)	SAP IIB[j,k]	Lys-Leu-Cys-Pro-Gly-Gly-Asn-Cys-Val	6	↑cAMP, ↑cGMP
Clypeaster japonicus (sand dollar)	Mosact (SAP III)[l,m]	Asp-Ser-Asp-Ser-Ala-Gln-Asn-Leu-Ile-Gly	9	↑cAMP, ↑cGMP
Diadema setosum (sea urchin)	SAP IV[n]	Gly-Cys-Pro-Trp-Gly-Gly-Ala-Val-Cys	?	↑cAMP, ↑cGMP ↑Respiration
Brissus agassizii (heart urchin)	SAP V[n]	Gly-Cys-Glu-Gly-Leu-Phe-His-Gly-Met-Gly-Asn-Cys	?	↑cAMP, ↑cGMP
Montipora digitata (hard coral)	Compound 1	$CH_3(CH_2)_7C$-C-C-C-CH_2OH	?	Chemoattractant
	Compound 2	CH_3-C-$(CH_2)_7C$-C-C-C-CH_2OH	?	Sperm activator
	Compound 3[p]	CH_3-C-C-$C(CH_2)_4$-C-C-C-C-CH_2OH	?	
Limulus polyphemus (horseshoe crab)	SMI[q]	Unknown (M_r = 500–2000)	?	Initiates motility
Clupea pallasi (Pacific herring)	SMIF[r]	Unknown (M_r = 105,000)	?	Initiates motility



Speract Receptor

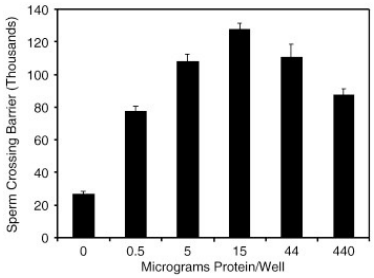
Speract

Progesterone at the picomolar range is a chemoattractant for mammalian spermatozoa

By means of a videomicroscopy system and a computer image analysis, we performed chemotaxis assays to detect true chemotaxis in human spermatozoa, in parallel to immunohistochemistry detection of progesterone inside the cumulus cells. Progesterone indeed chemotactically guides mammalian spermatozoa at very low hormone concentrations, and the cumulus oophorus could be a potential place for sperm chemotaxis mediated by progesterone in vivo. (Fertil Steril® 2006;86:745–9. ©2006 by American Society for Reproductive Medicine.)

Xenopus tropicalis allurin: Expression, purification, and characterization of a sperm chemoattractant that exhibits cross-species activity

Lindsey A. Burnetta, Serenity Boylesa, Christopher Spencera, Allan L. Biebera and Douglas E. ChandlerCorresponding Author Contact Information, a, E-mail The Corresponding Author
aMolecular and Cellular Biology Program, School of Life Sciences and Department of Chemistry and Biochemistry, Arizona State University, Tempe, AZ 85287-4501, USA
Received 19 December 2007; revised 30 January 2008; accepted 31 January 2008. Available online 15 February 2008.



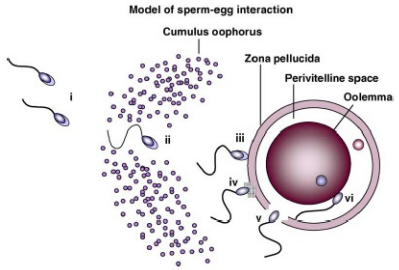


Fig. 1. Schematic diagram of the fertilization process. Within the female reproductive tract (i), sperm undergo a series of surface and intracellular transformations, collectively termed capacitation, which enables them to penetrate the cumulus oophorus (ii), bind to the zona pellucida (ZP) (iii), and undergo the acrosome reaction (iv). The release of hydrolytic enzymes from the acrosome facilitates sperm passage through the ZP (v), and fusion with the oolemma (vi).

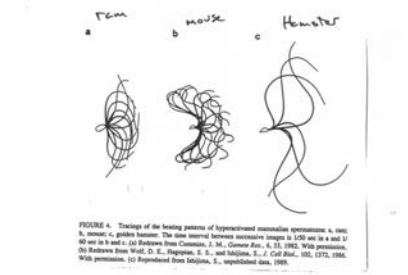
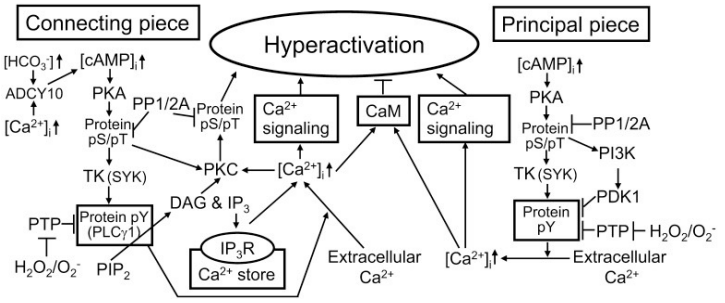


FIGURE 4. Tracings of the beating patterns of hyperactivated mammalian spermatozoa: a, ram; b, mouse; c, golden hamster. The time interval between successive images is 1/30 sec in a and 1/60 sec in b and c. (a) Redrawn from Cummins, J. M., *Gamete Res.*, 6, 33, 1982. With permission. (b) Redrawn from Wolf, D. E., Hagopian, S. S., and Ishijima, S., *J. Cell Biol.*, 102, 1372, 1986. With permission. (c) Reproduced from Ishijima, S., unpublished data, 1989.

TABLE 4. *The ability of acrosome-reacted guinea-pig spermatozoa to fertilize zona-intact and zona-free eggs[a]*

Age (hours) of acrosome-reacted sperm at insemination	% Acrosome-reacted sperm (in entire population) which are		% Eggs fertilized	
	Motile	Hyperactivated	Zona-intact	Zona-free
1	100	95	100	—
2	96	66	88	—
3	92	39	33	—
4	90	6	0	100
6	57	0	0	—
8	—	—	—	94
10	0	0	—	0

[a]From Flemming and Yanagimachi (159). Guinea-pig spermatozoa were induced to undergo a synchronous acrosome reaction. A population of 100% acrosome-reacted spermatozoa was isolated and incubated for up to 10 hr before they were mixed with zona-intact or zona-free guinea-pig eggs. Note that (a) there is a close correlation between hyperactivated motility of spermatozoa and the ability of spermatozoa to fertilize zona-intact eggs (to cross the zona) and (b) spermatozoa retain their ability to fuse with zona-free eggs for many hours after losing their ability to cross the zona.



Possible segment-specific cAMP signal transductions regulating transition of the flagellar movement pattern to hyperactivation in boar spermatozoa. ADCY10, adenylyl cyclase 10; cAMP, cyclic adenosine 3´,5´-monophosphate; PKA, protein kinase A (cAMP-dependent protein kinase); pS/pT, serine/threonine phosphorylation; PP, protein phosphatase; TK, tyrosine kinase; SYK, spleen tyrosine kinase; PTP, protein tyrosine phosphatase; pY, tyrosine phosphorylation; PLC, phospholipase C; PIP2, phosphatidylinositol 4,5-bisphosphate; DAG, 1,2-diacylglycerol; IP3, inositol 1,4,5-trisphosphate; IP3R, IP3 receptor; PKC, protein kinase C; PI3K, phosphatidylinositol-3 kinase; PDK1, phosphoinositide-dependent protein kinase-1; CaM, calmodulin.

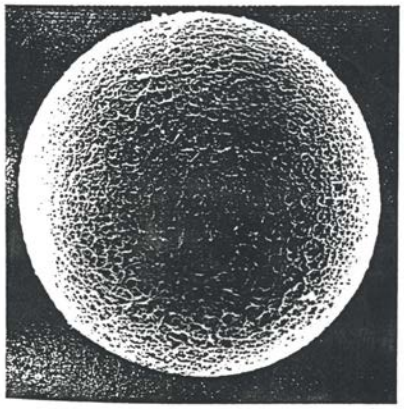


Figure 1–3. Hamster zona pellucida. The surface of the zona pellucida exhibits a fenestrated, multilayered appearance. ×1500. *(Reproduced, with permission, from Phillips, DM, et al.* J Exp Zool. *213:1, 1980.)*

Current Biology Vol 14 No 17
R692

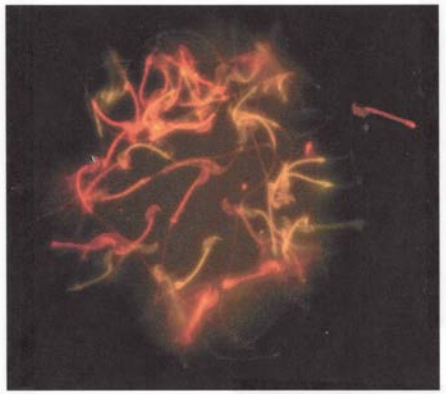


Figure 1. Mouse sperm labeled with two fluorescent dyes bound to the egg coat.

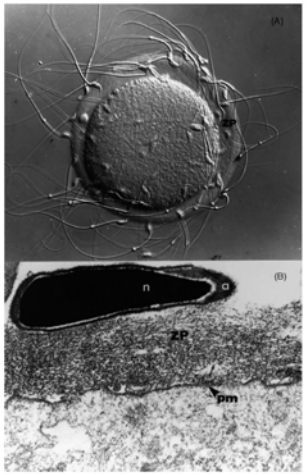


Fig. 1. Binding of free-swimming mouse sperm to the ZP of ovulated mouse eggs. (A) Photomicrograph (Nomarski differential interference contrast) of sperm bound to the ZP of an ovulated egg. (B) Transmission electron micrograph of a sperm head bound to the ZP of an ovulated egg. Zona pellucida (ZP), nucleus (n), acrosome (a), plasma membrane (PM).

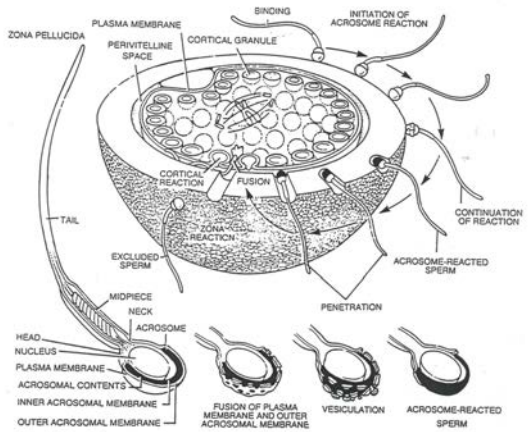
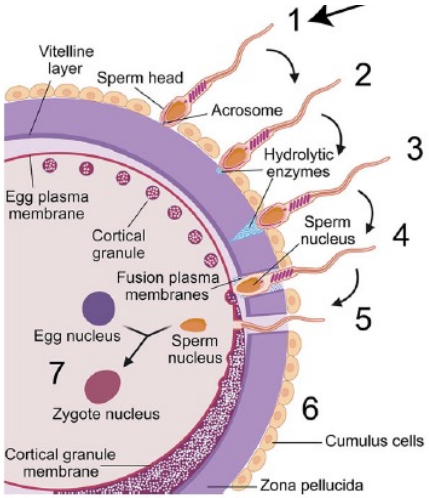
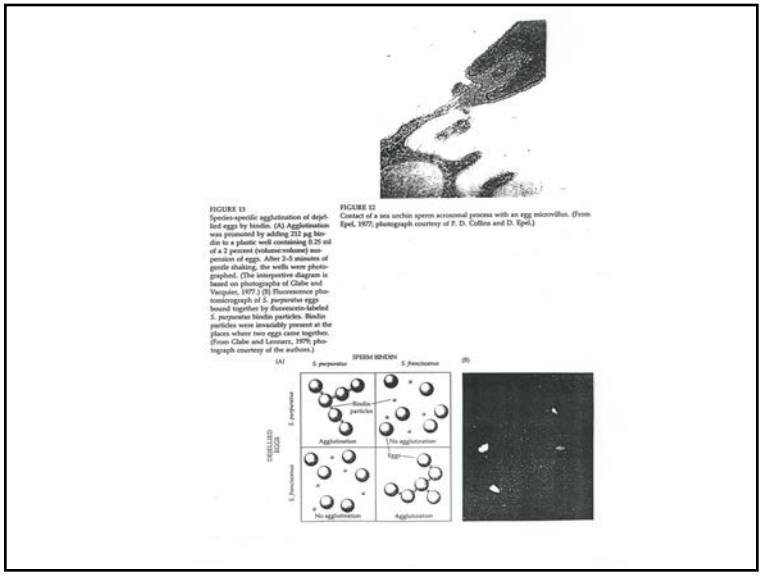


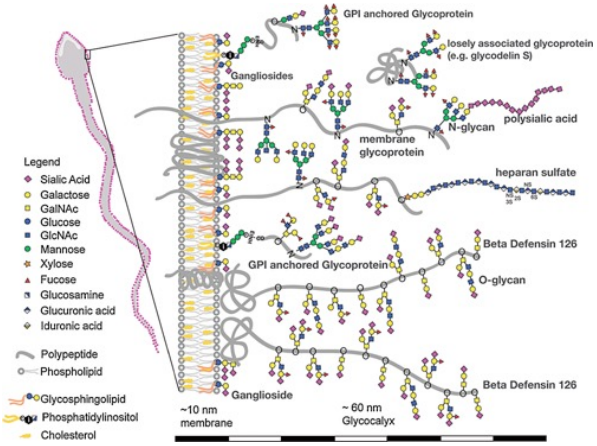
Figure 1–5. Fertilization pathway. After the spermatozoa binds to the zona pellucida, the acrosome reaction takes place (see detail at the bottom). The outer membrane of the acrosome fuses at several points with the plasma membrane surrounding the sperm head. Those fused membranes form vesicles that are eventually sloughed from the head, exposing the proteolytic enzymes of the acrosomal granule. The enzymes digest a pathway through the zona pellucida, enabling the sperm to advance to the egg surface. Eventually, the sperm fuses with the egg membrane, completing the fertilization process. This fusion triggers the cortical and zona reactions. The zona as a result of enzyme modification becomes impermeable to further sperm penetration and polyspermy. (Reproduced, with permission, from Wassarman PM. Sci Am. *259: 1988.)*

The cell biology of fertilization: Gamete attachment and fusion
Siu KK, Serrão VHB, Ziyyat A, Lee JE.
J Cell Biol. 2021 Oct 4;220(10):e202102146.

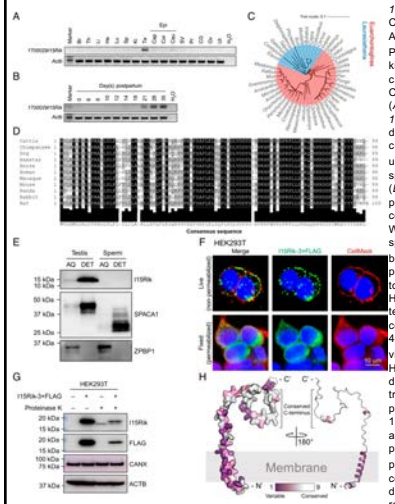




Sugar-coated sperm: Unraveling the functions of the mammalian sperm glycocalyx.
Tecle E, Gagneux P.
Mol Reprod Dev. 2015 Sep;82(9):635-50.

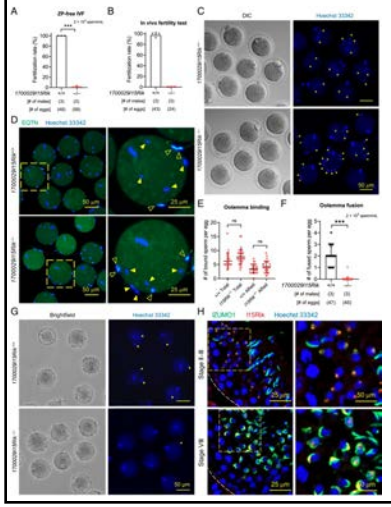


1700029I15Rik orchestrates the biosynthesis of acrosomal membrane proteins required for sperm-egg interaction
Lu Y, Shimada K, Tang S, et al.
Proc Natl Acad Sci U S A. 2023 Feb 21;120(8):e2207263120.



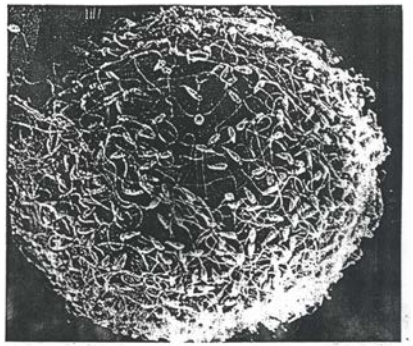
1700029I15Rik Is a Testis-Enriched Type-II Transmembrane Protein Conserved in Mammals and Expressed during Spermiogenesis. (*A*) Analysis of *1700029I15Rik* expression in various mouse tissues by RT-PCR. Br, brain; Th, thymus; Li, liver; He, heart; Lu, lung; Sp, spleen; Ki, kidney; Te, testis; Cap, caput epididymis; Cor, corpus epididymis; Cau, cauda epididymis; Epi, epididymis; SV, seminal vesicle; Pr, prostate; CG, coagulating gland; Ov, ovary; Ut, uterus. The expression of *β-actin* (*Actb*) was analyzed as a loading control. (*B*) Analysis of *1700029I15Rik* expression in mouse testes during postnatal development. (*C*) Phylogenetic tree depicting the evolutionary conservation of 1700029I15Rik in mammals. The tree was visualized using the interactive Tree of Life (iTOL) (16). Red and blue highlighted species belong to Euarchontoglires and Laurasiatheria, respectively. (*D*) Multiple sequence alignment of 1700029I15Rik orthologous proteins in 11 mammalian species. The *Lower* panel indicates the consensus sequence and the extent of amino acid conservation. (*E*) Western blot detection of 1700029I15Rik (I15Rik) in mouse testes and sperm fractionated by Triton X-114. SPACA1 and zona pellucida binding protein 2 (ZPBP1) were analyzed as positive controls for the proteins enriched in the AQ and DET phases, respectively. (*F*) In vitro topological analysis of 1700029I15Rik by live cell immunostaining. HEK293T cells were transiently transfected with a plasmid encoding C-terminal 3 × FLAG-tagged 1700029I15Rik. Live or fixed HEK293T cells were probed with an anti-FLAG antibody and an Alexa Fluor™ 488-conjugated secondary antibody. Cell membranes and nuclei were visualized by CellMask™ deep red plasma membrane stain and Hoechst 33342, respectively. (*G*) In vitro proteinase K protection assay depicting the topology of 1700029I15Rik. Live HEK293T cells transiently expressing 1700029I15Rik-3 × FLAG were treated with proteinase K and subjected to protein extraction. The levels of 1700029I15Rik-3 × FLAG before and after the enzyme treatment were analyzed by Western blotting. CANX and ACTB were analyzed in parallel as loading controls. (*H*) 1700029I15Rik protein structure predicted by AlphaFold (AF-Q8CF31-F1) (17). The degree of residue conservation was determined by ConSurf (18) and plotted to the three-dimensional structures, with purple representing variable and white representing conserved.

1700029I15Rik orchestrates the biosynthesis of acrosomal membrane proteins required for sperm-egg interaction
Lu Y, Shimada K, Tang S, et al.
Proc Natl Acad Sci U S A. 2023 Feb 21;120(8):e2207263120.

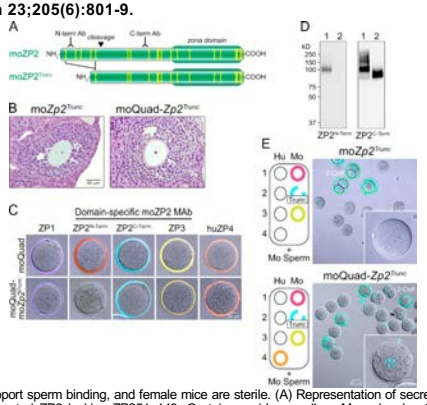


Infertility of *1700029I15Rik* Knockout Males Is Attributed to Impaired Sperm–Egg Interaction. (*A*) In vitro fertilization (IVF) analysis of sperm fertilizing ability using wild-type ZP-free eggs. (*B* and *C*) In vivo fertility test of wild-type and *1700029I15Rik*$^{-/-}$ males. Eggs were harvested from superovulated B6D2F1 female mice that had copulated with wild-type or *1700029I15Rik* knockout males. Sperm in the perivitelline space (yellow arrowheads) and pronuclei in the fertilized eggs (yellow asterisks) were visualized by Hoechst 33342. (*D* and *E*) In vitro analysis of sperm–egg binding. Spermatozoa pre-incubated in the Toyoda, Yokoyama, Hoshi (TYH) medium were probed with an anti-EQTN antibody and an Alexa Fluor™ 488-conjugated secondary antibody to reveal the acrosomal status. The acrosome-intact and acrosome-reacted sperm are marked by solid and hollow arrowheads, respectively. Sperm heads were stained with Hoechst 33342. (*F* and *G*) In vitro analysis of sperm–egg fusion using Hoechst 33342-preloaded ZP-free eggs. Yellow arrows indicate the fused sperm heads carrying the Hoechst dye transferred from the eggs. (*H*) Coimmunostaining of IZUMO1 (green) and 1700029I15Rik (red) in wild-type testis cryosections. Cell nuclei were visualized by Hoechst 33342.

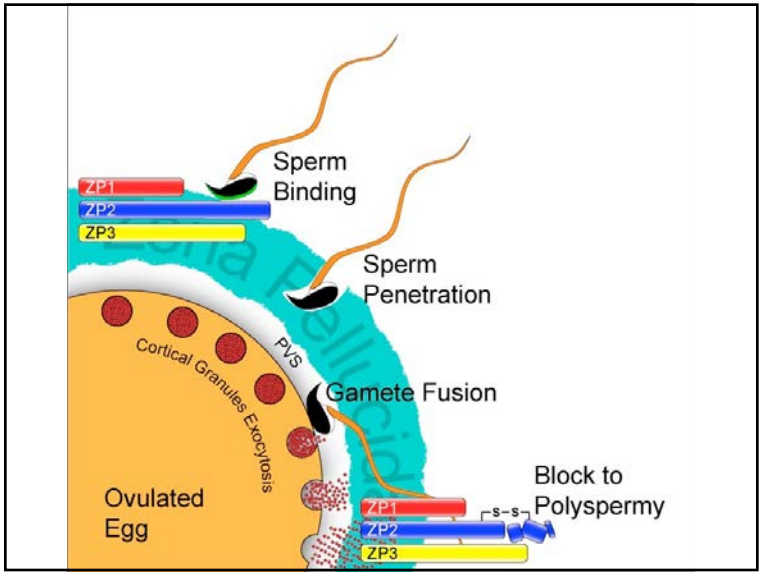
FIGURE 15
Scanning electron micrograph of sea urchin sperm bound to the vitelline envelope of an egg. (Photograph courtesy of C. Glabe, L. Perez, and W. J. Lennarz.)

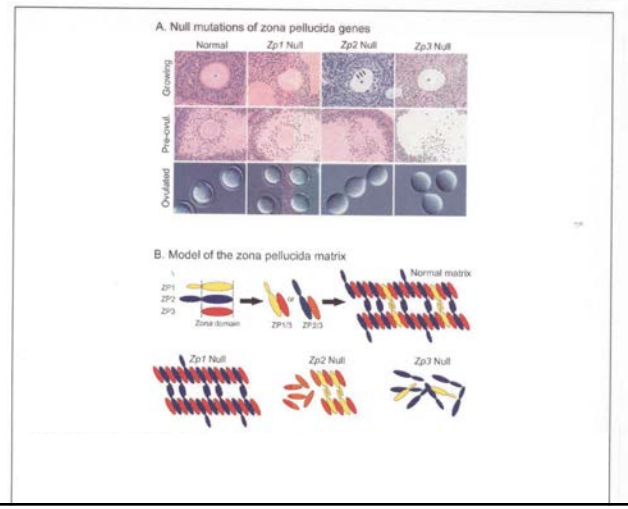


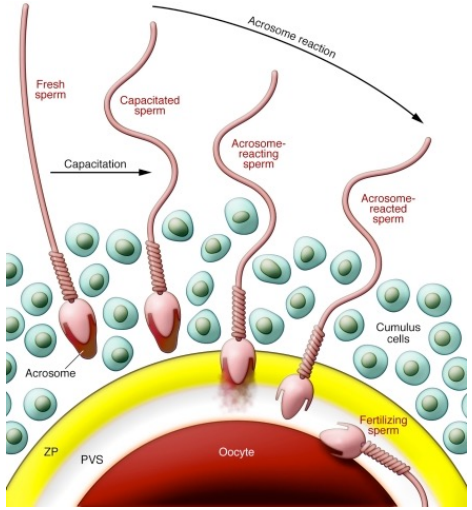
A single domain of the ZP2 zona pellucida protein mediates gamete recognition in mice and humans.
Avella MA, Baibakov B, Dean J.
J Cell Biol. 2014 Jun 23;205(6):801-9.



Truncated ZP2 does not support sperm binding, and female mice are sterile. (A) Representation of secreted ectodomains of normal mouse ZP235–633 and truncated ZP2 lacking ZP251–149. Cysteine residues, yellow. Monoclonal antibodies that bind N and C terminal to the postfertilization cleavage site (arrowhead) and zona domains are indicated above. (B) Ovarian histology of moZp2Trunc and moQuad-Zp2Trunc transgenic mice in Zp2Null background as in Fig. 1 C. (C) moQuad(huZP4) and moQuad-Zp2Trunc eggs stained with domain-specific monoclonal antibodies as in Fig. 1 D. (D) Immunoblot of eggs (15) from moQuad(huZP4) (1) and moQuad-Zp2Trunc (2) mice stained with domain-specific monoclonal antibodies. Molecular masses are indicated on the left. (E) Mouse sperm binding to Zp2Trunc and moQuad-Zp2Trunc eggs as in Fig. 1 E.







Mechanism of sperm-egg interaction.
Over the nucleus of each mammalian sperm is a membranous sac known as the acrosome, which is filled with many kinds of hydrolytic enzymes. In the female reproductive tract or in an IVF medium, sperm undergo capacitation, which permits the acrosome reaction. Near the eggs, probably stimulated by the cumulus cells and the ZP, sperm release their acrosomal contents by exocytosis and penetrate the ZP. Only acrosome-reacted sperm fuse with eggs, but their competency for fusion does not last long. Cumulus cells are packed together by hyaluronic acid at ovulation and become diffuse during fertilization. PVS, perivitelline space.

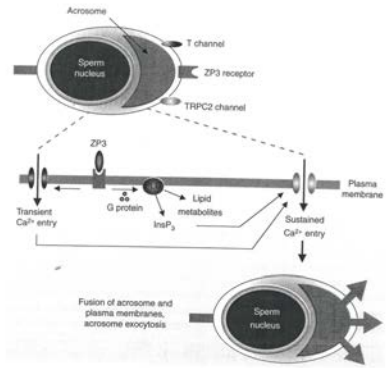


Figure 2 **Triggering of the acrosome reaction.** A model of the events involved in ZP3 signal transduction is shown. In the sperm head, TRPC2–ZP3 receptor activation during adhesion to the zona pellucida results in calcium entry through T-type channels, causing a transient elevation of cytosolic calcium concentration and activation of PLC through a G_q and/or G_s protein-mediated pathway, resulting in the production of $InsP_3$ and diacylglycerol. PLC and the transient calcium elevation function in concert to produce persistent calcium entry through a TRPC2 channel that directly drives the acrosome reaction, triggering fusion of the outer acrosome membrane with the plasma membrane and releasing acrosomal contents. See text for details.

Discrete Dynamic Model of the Mammalian Sperm Acrosome Reaction: The Influence of Acrosomal pH and Physiological Heterogeneity
Aldana A, Carneiro J, Martínez-Mekler G, Darszon A.
Front Physiol. 2021 Jul 19;12:682790.

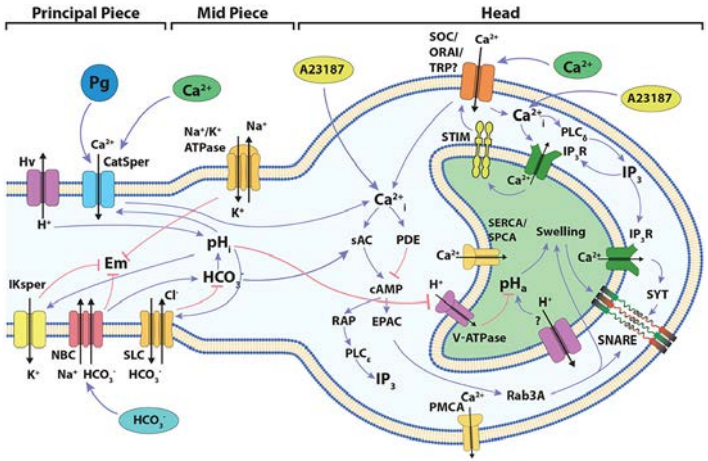


TABLE 2
SUMMARY OF CHARACTERIZED ACROSOME REACTION-INDUCING MOLECULES AND THEIR EFFECTS ON SPERM

Species	Name	Structure	Actions
Sea urchin	FSG[a]	Fucose sulfate glycoconjugate	$\uparrow Ca^{2+}$, $\uparrow Na^+$ $\uparrow H^+$, K^+ release $\uparrow pH_i$ $\uparrow$Adenylyl cyclase $\uparrow$cAMP $\uparrow$Protein kinase A $\uparrow IP_3$ $\uparrow$Phospholipase D $\uparrow$Phosphatidate
Starfish	ARIS[b]	Fucose sulfate glycoconjugate	$\uparrow Ca^{2+}$, $\uparrow Na^+$ $\uparrow H^+$, K^+ release $\uparrow pH_i$; $\uparrow$cAMP (only in presence of CoARIS)
	CoARIS[b]	Sulfated steroidal saponins	Cofactor for ARIS
Mouse	ZP3[c]	Glycoprotein	G_i activation $\uparrow Ca^{2+}$ $\uparrow pH_i$ $\uparrow$cAMP

[a] SeGall and Lennarz, 1979, 1981; Garbers and Kopf, 1980; Garbers *et al.*, 1983; Trimmer and Vacquier, 1986.
[b] Ikadai and Hoshi, 1981a,b; Matsui *et al.*, 1986a,b; Nishiyama *et al.*, 1987a; Hoshi *et al.*, 1990.
[c] Wassarman, 1988, 1990; Kopf and Gerton, 1990; Florman and Babcock, 1990.

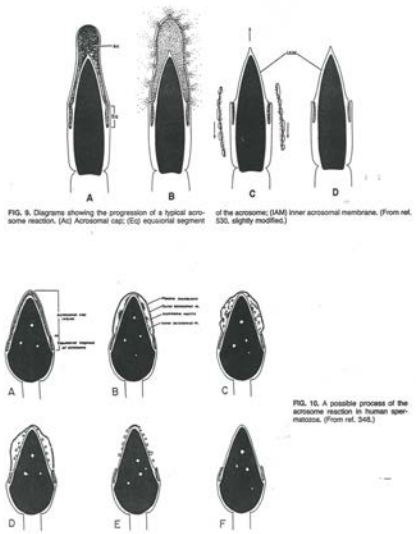


FIG. 9. Diagrams showing the progression of a typical acrosome reaction. (Ac) Acrosomal cap; (Eq) equatorial segment of the acrosome; (IAM) inner acrosomal membrane. (From ref. 530, slightly modified.)

FIG. 10. A possible process of the acrosome reaction in human spermatozoa. (From ref. 348.)

TABLE 2. *Enzymes reported to be of acrosomal origin*

First reported before 1980	First reported after 1980 (references)
Hyaluronidase	β-*N*-Acetylhexosaminidase (454)
Acrosin	β-Galactosidase (454)
Proacrosin	β-Glucuronidase (454)
Acid proteinase	α-L-Fucosidase (454)
Esterase	Phospholipase C (453)
Neuraminidase	Cathepsin D (456)
Phosphatase	Peptidyl peptidase (471b)
Phospholipase A	Ornithin decarboxylase (400)
β-*N*-acetylglucosaminidase	
Arylsulfatase	
Arylamidase	
Collagenase	

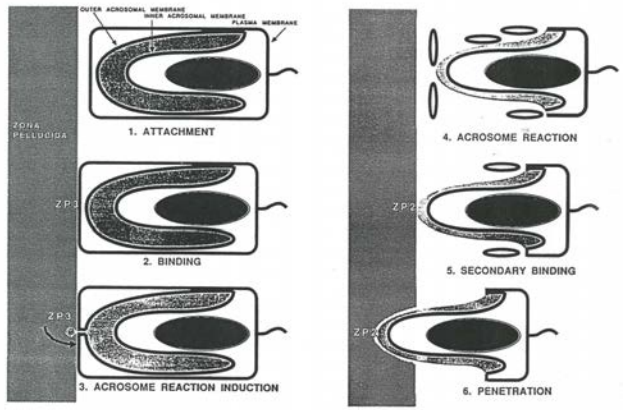
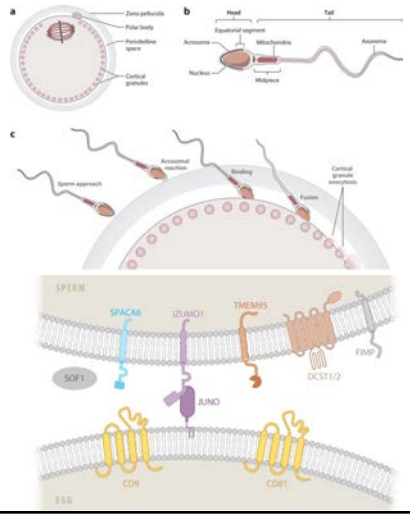


FIGURE 1. Sequence of interactions between the sperm head and the egg's zona pellucida.

The Fertilization Enigma: How Sperm and Egg Fuse
Deneke VE, Pauli A.
Annu Rev Cell Dev Biol. 2021 Oct 6;37:391-414.



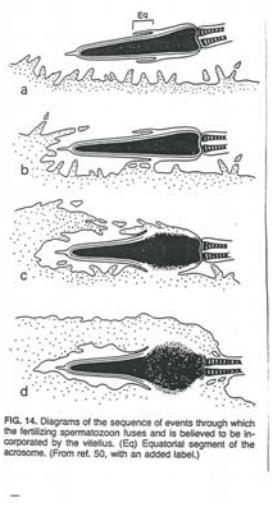
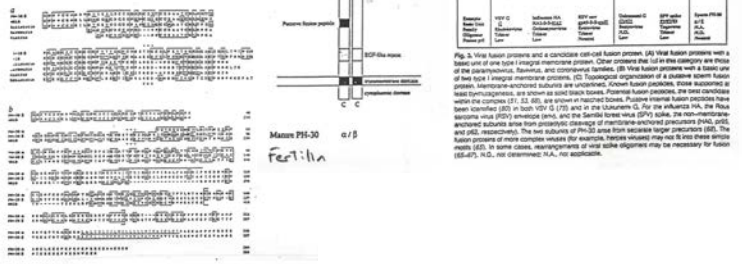


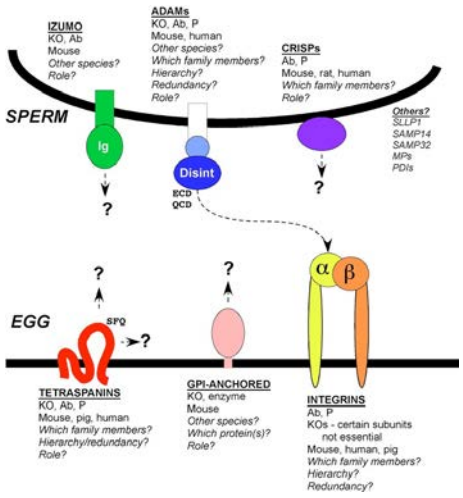
FIG. 14. Diagrams of the sequence of events through which the fertilizing spermatozoon fuses and is believed to be incorporated by the vitellus. (Eq) Equatorial segment of the acrosome. (From ref. 50, with an added label.)

A POTENTIAL FUSION PEPTIDE AND AN INTEGRIN LIGAND DOMAIN IN A PROTEIN ACTIVE IN SPERM-EGG FUSION.

Blobel CP, Wolfsberg TG, Turck CW, Myles DG, Primakoff P, White JM.

Department of Pharmacology, University of California San Francisco.





Evolutionarily conserved sperm factors, DCST1 and DCST2, are required for gamete fusion.
Inoue N, Hagihara Y, Wada I.
Elife. 2021 Apr 19;10:e66313.

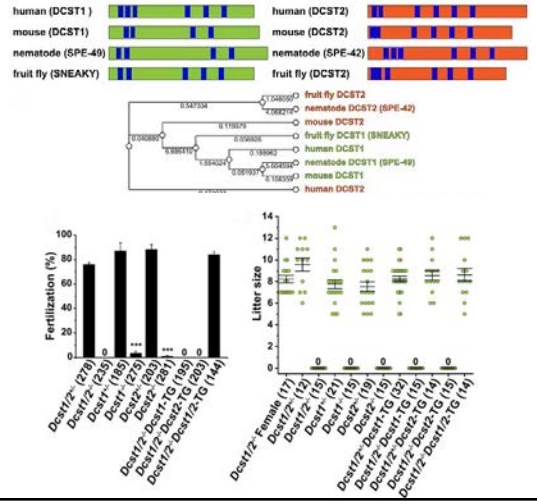


Table 1. **Cellular protein factors involved in sperm–egg attachment or fusion**

Protein	Year identified	Role in fertilization	Structural features	References
CD9	1999	CD9 is expressed on the surface of the oocyte and accumulates during the attachment event; it may modulate the integrity of the oocyte membrane; its precise role in sperm–egg fusion remains unclear	CD9 is a tetraspanin with four transmembrane domains and two extracellular loops (short and long)	Miyado et al., 2000; Le Naour et al., 2000; Kaji et al., 2000; Chen et al., 1999; Umeda et al., 2020; Zimmerman et al., 2016; Zhang and Huang, 2012; Dahmane et al., 2019; Runge et al., 2007; Zhu et al., 2002; Chalbi et al., 2014; Rubinstein et al., 2006; Ziyyat et al., 2006
IZUMO1	2005	IZUMO1 relocates to the equatorial region of the sperm head after the acrosome reaction; high-affinity binding of IZUMO1 to JUNO results in initial attachment of sperm and egg in the PVS	The protein has an N-terminal 4HB, followed by a β-hinge and an IgSF domain; the structure is stabilized by five disulfide bonds	Inoue et al., 2005; Ellerman et al., 2009; Young et al., 2015; Satouh et al., 2012; Aydin et al., 2016; Ohto et al., 2016; Nishimura et al., 2016; Kato et al., 2016
JUNO	2014	JUNO is expressed on the surface of the oocyte membrane and serves as the receptor of IZUMO1	JUNO has structural similarity to folate receptors; it is a globular α/β protein composed of five α helices, three 3_{10} helices, and four short β strands stabilized by eight disulfide bonds	Bianchi et al., 2014; Kato et al., 2016; Han et al., 2016; Jean et al., 2019; Yamaguchi et al., 2007; Aydin et al., 2016; Ohto et al., 2016
SPACA6	2014	SPACA6 is expressed in sperm and localized to the equatorial segment after the acrosome reaction, but its specific role in sperm–egg fusion remains unknown	The three-dimensional structure of SPACA6 is currently unknown; SPACA6 is similar in organization to IZUMO1 with a signal peptide, followed by an α-helical domain, an IgSF domain, a transmembrane helix, and a cytoplasmic tail	Lorenzetti et al., 2014; Noda et al., 2020; Barbaux et al., 2020
TMEM95	2014	TMEM95 is localized to the equatorial segment of sperm and is essential for sperm–egg fusion and male fertility in mice, but its specific role in sperm–egg fusion is currently unknown	The structure of TMEM95 is currently unknown; TMEM95 consists of a signal peptide, an N-terminal helix-rich region, a transmembrane helix, and a leucine-rich cytoplasmic domain	Rausch et al., 2014; Zhang et al., 2016; Noda et al., 2020; Fernandez-Fuertes et al., 2017; Lamas-Toranzo et al., 2020
SOF1	2020	SOF1 is predicted to be a secreted factor essential for fusion; its role is still not fully understood	No structural information to date; primary sequence analysis revealed the presence of conserved LLLL and CFNLAS motifs	Noda et al., 2020
FIMP	2020	FIMP is involved in sperm–egg fusion; only the transmembrane form is important in fertilization, but its role is still not fully determined	No structural information to date	Fujihara et al., 2020
DCST1/ DCST2	2021	DCST1 and DCST2 are involved in sperm–egg fusion; stability of SPACA6 is regulated by DCST1/2; DCST1/DCST2 are evolutionary conserved in vertebrates and invertebrates	No structural information to date; contains six putative transmembrane helices	Inoue et al., 2021

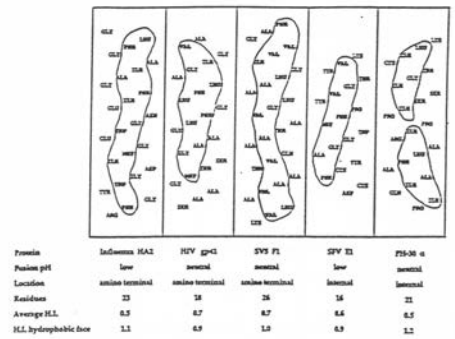


Fig. 4. Characteristics of the fusion peptides of several viral fusion proteins and a potential fusion peptide from a candidate sperm fusion protein. The sequences analyzed encompass those from the first to the last residue of the hydrophobic face (encircled residues) of the displayed helices. Bulky hydrophobic residues (H.I. ≥0.64; Ile, Phe, Val, Leu, Trp, Met) are in bold. Hydrophobicity indices (H.I.) were calculated with the normalized consensus scale of Eisenberg (53). Helices were plotted by using the program HELQ from R. Stroud.

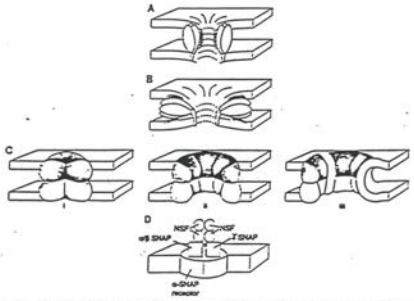
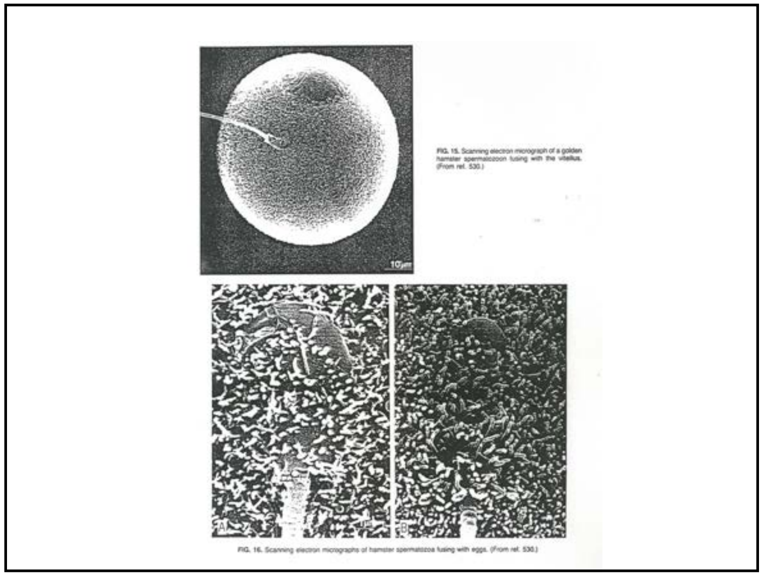
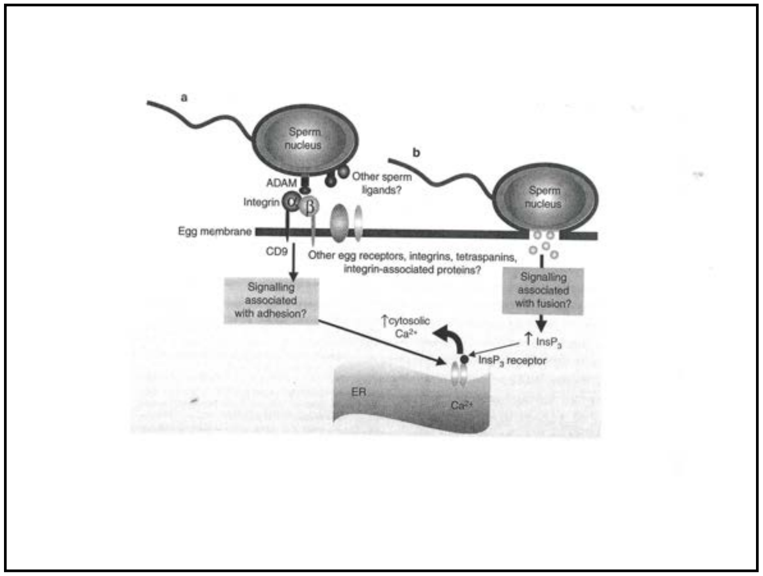
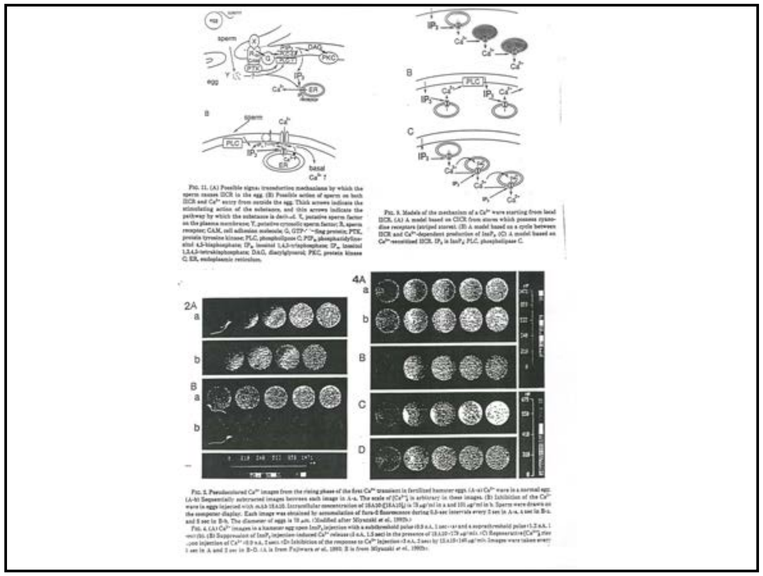


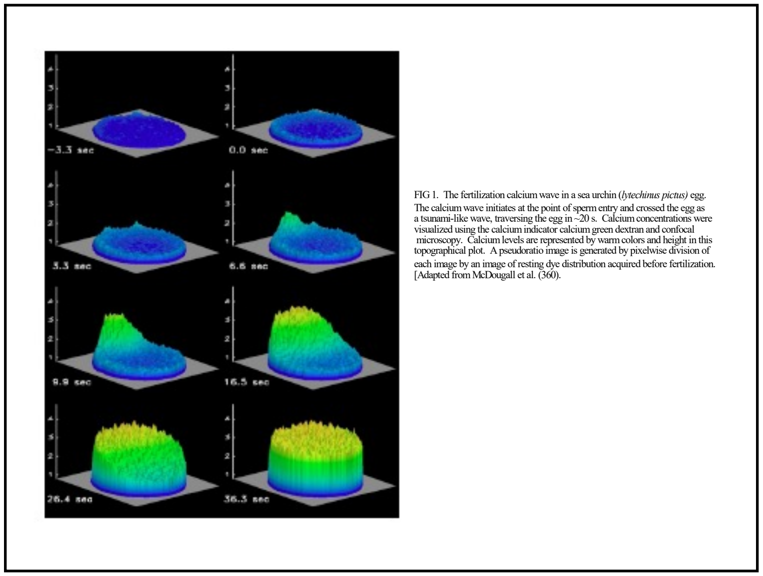
Fig. 2. Models of fusion pores and a fusion machine. (A) A model for an HA fusion pore lined with several upright HA trimers. The exposed fusion peptides projecting into the pore are thought to promote lipid mixing (*21*, *51*, *52*). (B) A model for an HA fusion pore lined with several tilted HA trimers. The exposed fusion peptides are thought to bind to both the viral and target membranes, bringing them into close apposition (*11*). (C) A model for the exocytic fusion pore formed by paired integral membrane multimeric proteins in the vesicle and plasma membranes (*49*, *50*). (i) The pore is closed. (ii) The pore opens. (iii) The pore dilates. (D) Minimal components of the NSF-containing fusion machine (*32*, *45*, *46*). It is not yet known whether γ-SNAP binds to the α-SNAP receptor or to a different molecule.

SCIENCE • VOL. 258 • 6 NOVEMBER 1992

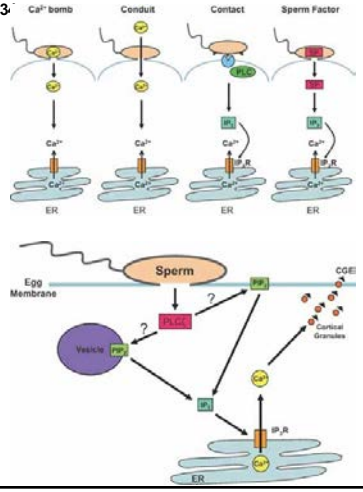

10µm
FIG. 15. Scanning electron micrograph of a golden hamster spermatozoon fusing with the vitellus. (From ref. 530.)
FIG. 16. Scanning electron micrographs of hamster spermatozoa fusing with eggs. (From ref. 530.)

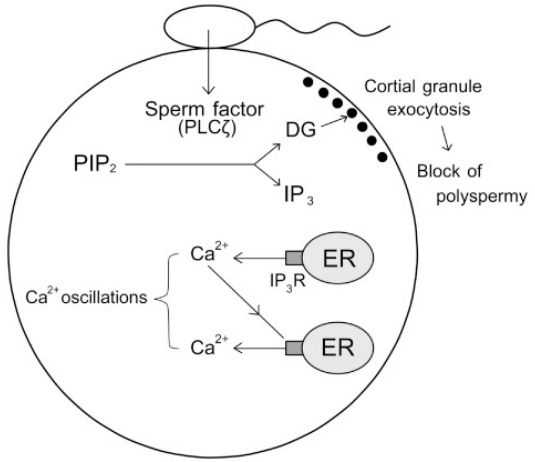

a
Sperm nucleus
ADAM
Other sperm ligands?
Integrin
β
b
Sperm nucleus
Egg membrane
CD9
Other egg receptors, integrins, tetraspanins, integrin-associated proteins?
Signalling associated with adhesion?
Signalling associated with fusion?
↑cytosolic Ca2+
↑ InsP3
InsP3 receptor
ER
Ca2+


2A
4A
B
C
D

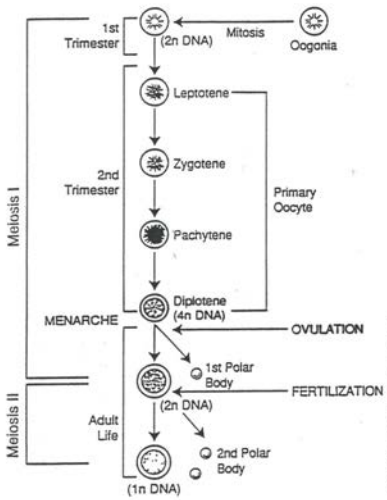

-3.3 sec
0.0 sec
3.3 sec
6.6 sec
9.9 sec
16.5 sec
26.4 sec
36.3 sec
FIG 1. The fertilization calcium wave in a sea urchin (*lytechinus pictus)* egg. The calcium wave initiates at the point of sperm entry and crossed the egg as a tsunami-like wave, traversing the egg in ~20 s. Calcium concentrations were visualized using the calcium indicator calcium green dextran and confocal microscopy. Calcium levels are represented by warm colors and height in this topographical plot. A pseudoratio image is generated by pixelwise division of each image by an image of resting dye distribution acquired before fertilization. [Adapted from McDougall et al. (360).

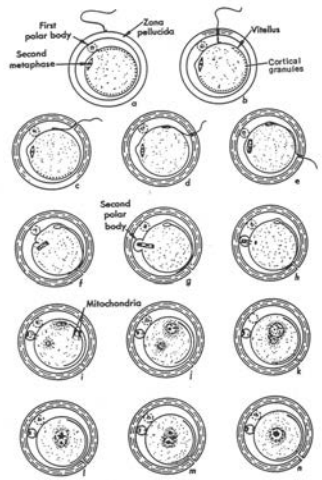
Starting a new life: sperm PLC-zeta mobilizes the Ca2+ signal that induces egg activation and embryo development: an essential phospholipase C with implications for male infertility.
Nomikos M, Swann K, Lai FA.
Bioessays. 2012 Feb;34(2):126-3

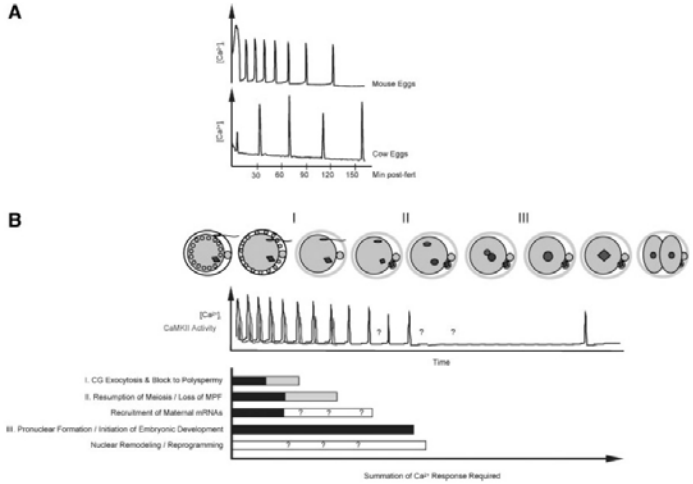


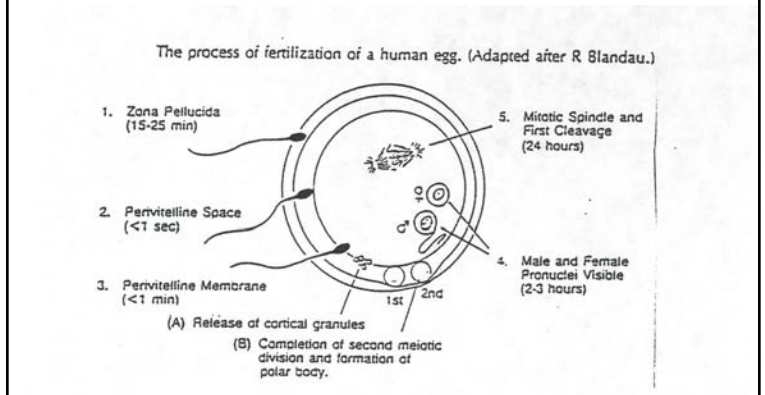


Model of oocyte activation induced by phospholipase C zeta (PLCζ). PLCζ was released from the sperm into ooplasm. PLCζ hydrolyzes phosphatidylinostitol (4,5)-bisphosphate (PIP2) into IP3 and diacyl glycerol (DG) and the IP3 leads to the release of Ca2+ from the endoplasmic reticulum (ER). Ca2+ induces amplitude of the Ca2+ release from ER, resulting in the induction of Ca2+ oscillations. Alternatively, Ca2+ oscillations depend upon Ca2+ induced IP3 production via PLCζ.









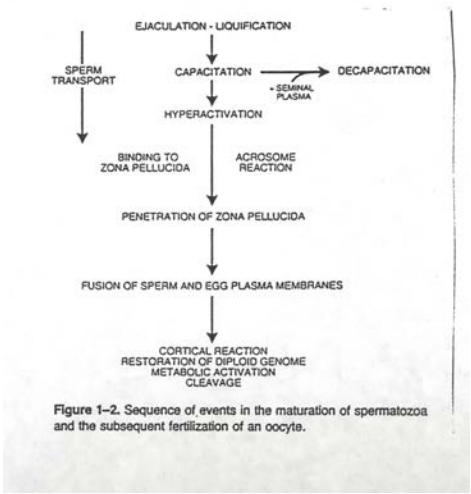
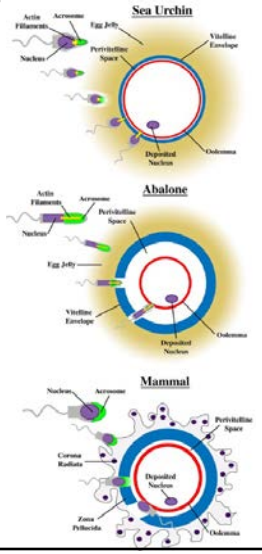


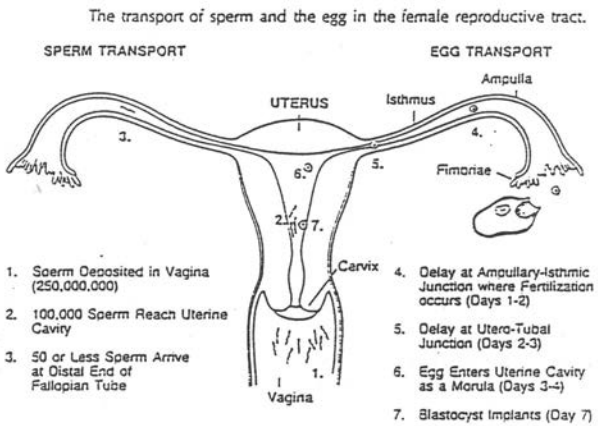
Figure 1–2. Sequence of events in the maturation of spermatozoa and the subsequent fertilization of an oocyte.

Molecular mechanisms and evolution of fertilization proteins
Carlisle JA, Swanson WJ.
J Exp Zool B Mol Dev Evol. 2021 Dec;336(8):652-665.

TABLE 1 Significant GRP models from sea urchins, abalone, and mammals

	Animal	Sperm	Egg	Positive selection?
Acrosome reaction	Sea urchin	su-REJ	FSP	Yes (su-REJ only)
	Abalone	?	?	?
	Mammal	PKDREJ	?	Yes
	Mammal	ZP3r/sp56	ZP3	Yes (ZP3 only)
Egg coat	Sea urchin	?	?	?
	Abalone	Lysin	VERL	Yes
	Mammal	?	ZP2	Yes
Oolemma adhesion/ fusion	Sea urchin	Bindin	EBR1	Yes
	Abalone	sp18	?	Yes
	Mammal	Izumo1	Juno	Yes
	Mammal	SLLP1	SAS1B	?





Preimplantation embryo

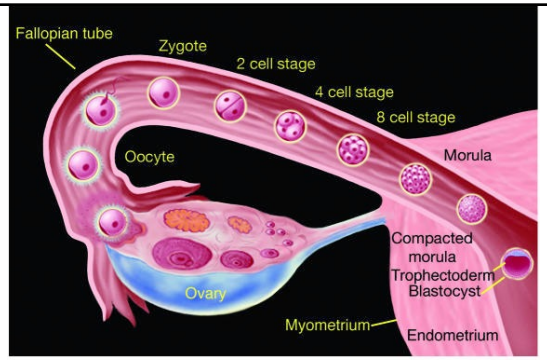
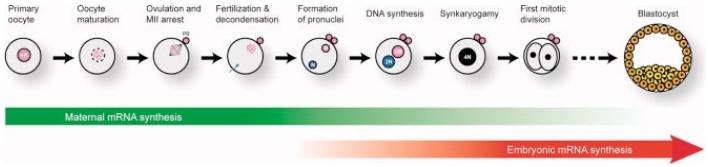
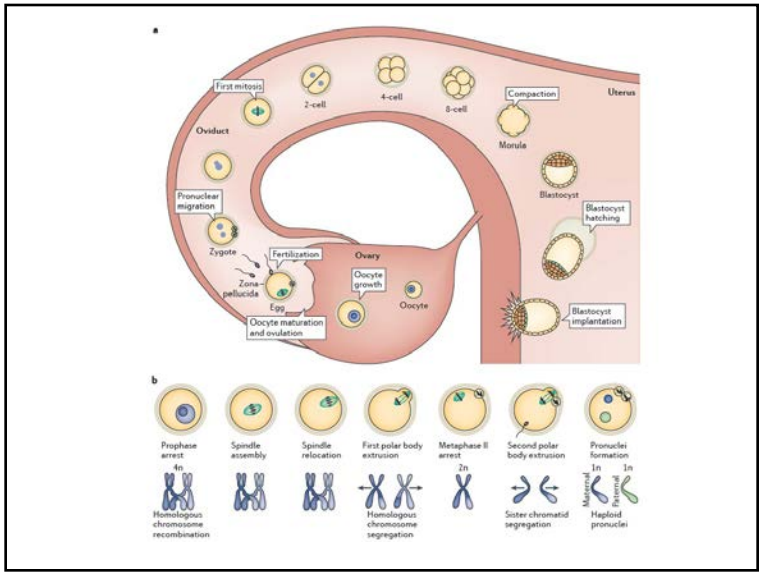
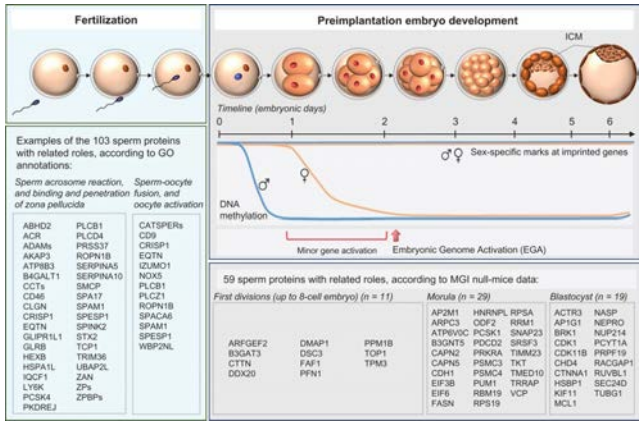


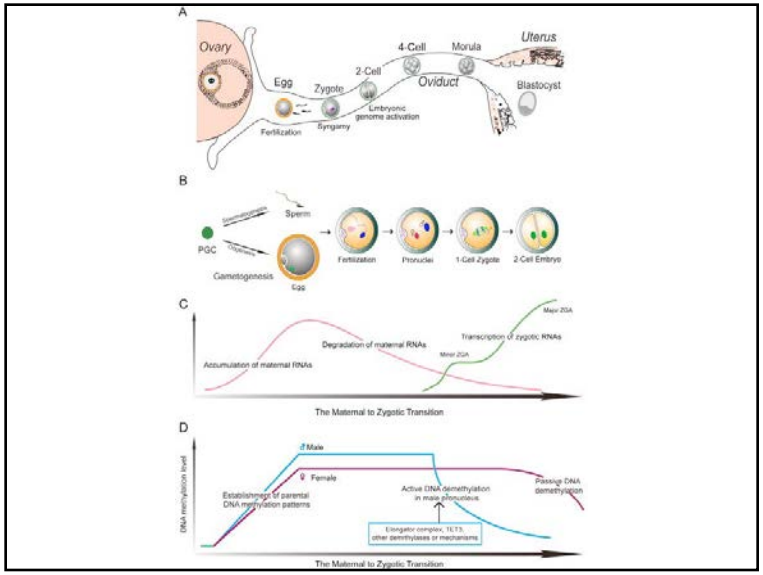
Figure 1.
The early stages of human development from fertilization to blastocyst formation. Fertilization occurs in the fallopian tube within 24 to 48 hours of ovulation. The initial stages of development, from fertilized ovum (zygote) to a solid mass of cells (morula), occur as the embryo passes through the fallopian tube encased within a nonadhesive protective shell (the zona pellucida). The morula enters the uterine cavity approximately two to three days after fertilization. The appearance of a fluid-filled inner cavity marks the transition from morula to blastocyst and is accompanied by cellular differentiation: the surface cells become the trophoblast (and give rise to extraembryonic structures, including the placenta) and the inner cell mass gives rise to the embryo. Within 72 hours of entering the uterine cavity, the embryo hatches from the zona, thereby exposing its outer covering of trophectoderm. Figure kindly provided by S.S. Gambhir and J. Strommer, Stanford University (Stanford, California, USA).

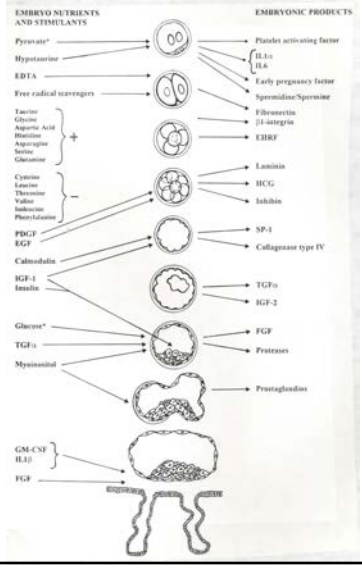


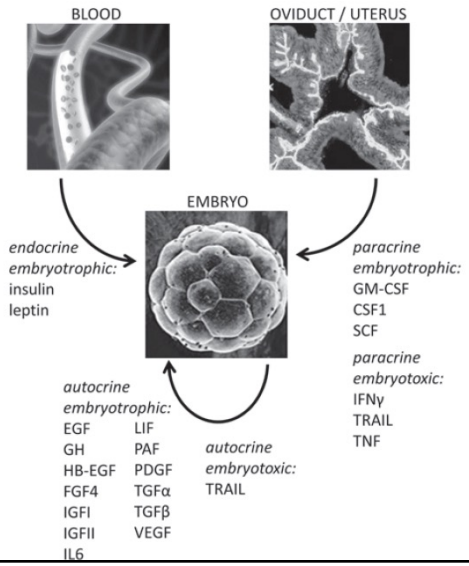
Summary of events in mammalian preimplantation development. Top: immature oogonia undergo DNA replication and pause in prophase of meiosis I as 4N primary oocytes. Completion of meiosis I results in extrusion of the first polar body (PB) and formation of a diploid secondary oocyte, which is released from the follicle upon ovulation. Thereafter, the second meiotic division is initiated and the secondary oocyte arrests in metaphase II (MII) until fertilization. Fertilization induces completion of meiosis II, producing the second polar body and a two-pronucleus zygote. The haploid pronuclei (paternal, blue; maternal, pink) migrate towards the center of the zygote and replicate their DNA content. Diploid pronuclei fuse during synkaryogamy and completion of the first mitotic division produces a two-cell zygote, which undergoes further cleavage divisions (not shown) to produce the blastocyst, which is comprised of the trophectoderm (orange) and the inner cell mass (yellow). Bottom: zygotic genome activation (represented in red) initiates during the two-pronucleus stage and is fully underway by the two-cell stage (see text). Maternal transcripts (represented in green) are gradually degraded and represent less than 10% of the total cellular transcripts by the two-cell stage.


a
First mitosis
2-cell
4-cell
8-cell
Compaction
Uterus
Oviduct
Morula
Blastocyst
Blastocyst hatching
Pronuclear migration
Zygote
Fertilization
Ovary
Zona pellucida
Oocyte growth
Egg
Oocyte
Oocyte maturation and ovulation
Blastocyst implantation
b
Prophase arrest
4n
Spindle assembly
Spindle relocation
First polar body extrusion
Metaphase II arrest
2n
Second polar body extrusion
Pronuclei formation
1n
1n
Maternal
Paternal
Homologous chromosome recombination
Homologous chromosome segregation
Sister chromatid segregation
Haploid pronuclei

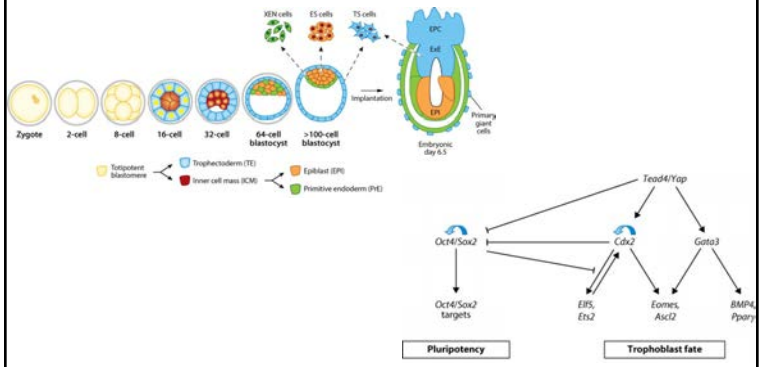

Fertilization
Preimplantation embryo development
ICM
Timeline (embryonic days)
0
1
2
3
4
5
6
Sex-specific marks at imprinted genes
DNA methylation
Minor gene activation
Embryonic Genome Activation (EGA)
Examples of the 103 sperm proteins with related roles, according to GO annotations:
Sperm acrosome reaction, and binding and penetration of zona pellucida
Sperm-oocyte fusion, and oocyte activation
ABHD2 ACR ADAMs AKAP3 ATP8B3 B4GALT1 CCTs CD46 CLGN CRISP1 EQTN GLIPR1L1 GLRB HEXB HSPA1L IQCF1 LY6K PCSK4 PKDREJ
PLCB1 PLCD4 PRSS37 ROPN1B SERPINA5 SERPINA10 SMCP SPA17 SPAM1 SPESP1 SPINK2 STX2 TCP1 TRIM36 UBAP2L ZAN ZPs ZPBPs
CATSPERs CD9 CRISP1 EQTN IZUMO1 NOX5 PLCB1 PLCZ1 ROPN1B SPACA6 SPAM1 SPESP1 WBP2NL
59 sperm proteins with related roles, according to MGI null-mice data:
First divisions (up to 8-cell embryo) (n = 11)
ARFGEF2 B3GAT3 CTTN DDX20
DMAP1 DSC3 FAF1 PFN1
PPM1B TOP1 TPM3
Morula (n = 29)
AP2M1 ARPC3 ATP6V0C B3GNT5 CAPN2 CAPN5 CDH1 EIF3B EIF6 FASN
HNRNPL ODF2 PCSK1 PDCD2 PRKRA PSMC3 PSMC4 PUM1 RBM19 RPS19
RPSA RRM1 SNAP23 SRSF3 TIMM23 TKT TMED10 TRRAP VCP
Blastocyst (n = 19)
ACTR3 AP1G1 BRK1 CDK1 CDK11B CHD4 CTNNA1 HSBP1 KIF11 MCL1
NASP NEPRO NUP214 PCYT1A PRPF19 RACGAP1 RUVBL1 SEC24D TUBG1


A
Ovary
Egg
Zygote
Fertilization
Syngamy
2-Cell
4-Cell
Morula
Oviduct
Uterus
Blastocyst
Embryonic genome activation
B
PGC
Gametogenesis
Sperm
Egg
Fertilization
Pronuclei
1-Cell Zygote
2-Cell Embryo
C
Major ZGA
Minor ZGA
Degradation of maternal RNAs
Transcription of zygotic RNAs
Accumulation of maternal RNAs
The Maternal to Zygotic Transition
D
Male
Female
Establishment of parental DNA methylation patterns
Active DNA demethylation in male pronucleus
Passive DNA demethylation
Elongator complex, TET3, other demethylases or mechanisms
DNA methylation level
The Maternal to Zygotic Transition


EMBRYO NUTRIENTS AND STIMULANTS
EMBRYONIC PRODUCTS
Pyruvate*
Hypotaurine
EDTA
Free radical scavengers
Taurine
Glycine
Aspartic Acid
Histidine
Asparagine
Serine
Glutamine
Cysteine
Leucine
Threonine
Valine
Isoleucine
Phenylalanine
PDGF
EGF
Calmodulin
IGF-1
Insulin
Glucose*
TGFα
Myoinositol
GM-CSF
IL1β
FGF
Platelet activating factor
IL1α
IL6
Early pregnancy factor
Spermidine/Spermine
Fibronectin
β1-integrin
EHRF
Laminin
HCG
Inhibin
SP-1
Collagenase type IV
TGFα
IGF-2
FGF
Proteases
Prostaglandins

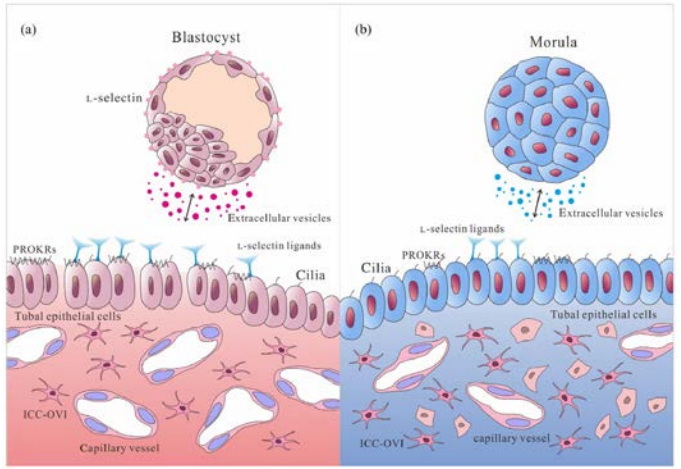


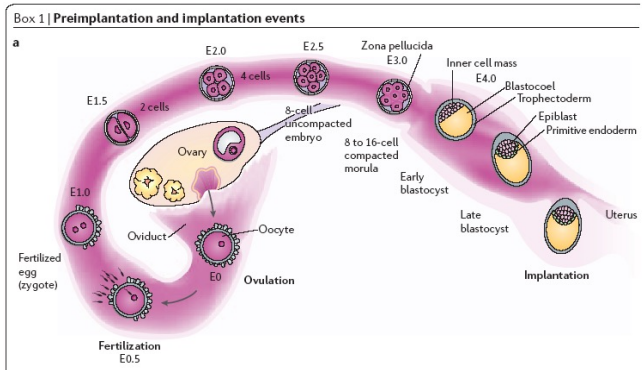
Genetic Control of Early Cell Lineages in the Mammalian Embryo.
Rossant J.
Annu Rev Genet. 2018 Nov 23;52:185-201.



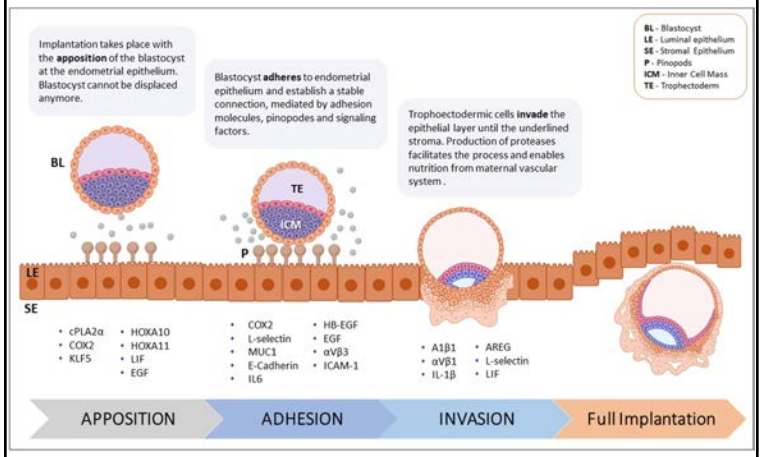
Implantation

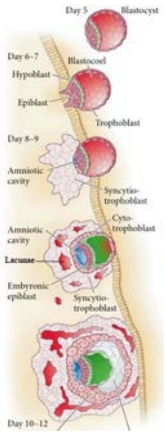
Interaction networks between the Fallopian tubes and the embryo in human tubal pregnancy: Current knowledge and perspectives.
Jiang H, Li JX.
J Obstet Gynaecol Res. 2021 Dec;47(12):4139-4147.



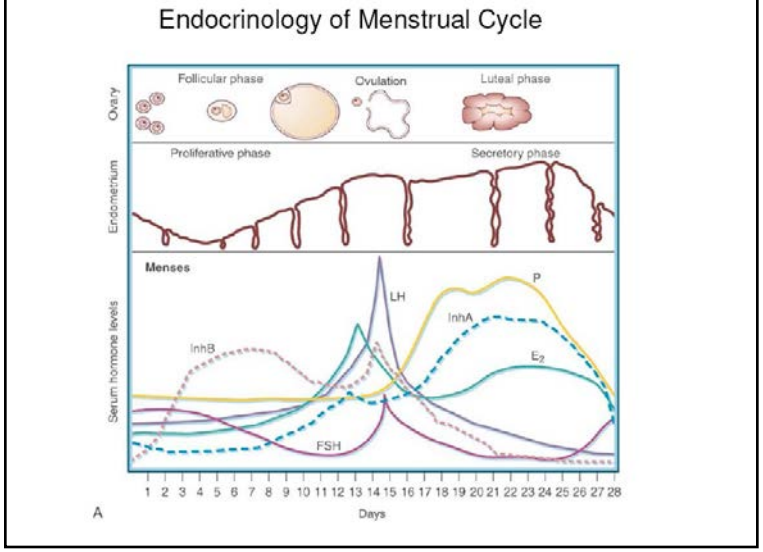


Main actors behind the endometrial receptivity and successful implantation.
Governini L, Luongo FP, Haxhiu A, Piomboni P, Luddi A.
Tissue Cell. 2021 Dec;73:101656.

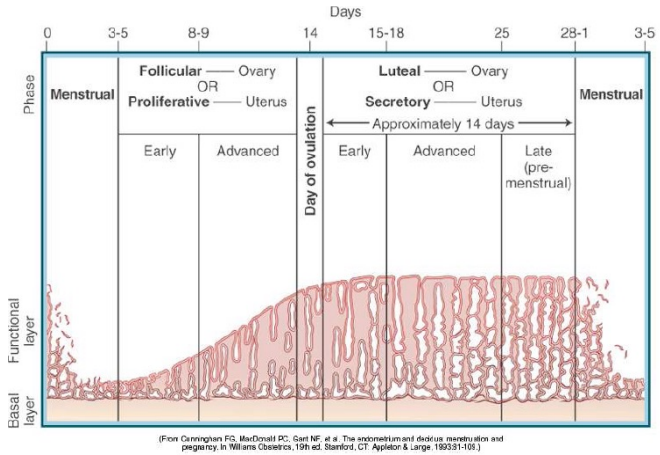




Morphological transition of the human conceptus during implantation. At day 6–7, a sub-population of TE at the polar end of the conceptus initiates attachment to uterine epithelial cells. Day 8–9, invasive STB penetrates the uterine epithelium, tunnels into the decidualizing endometrium, and begins to erode out lacunae (in red). The cytoTB layer (pink) between the embryo proper and the advancing syncytial mass continues to proliferate to replenish the STB. By day 12, the conceptus has penetrated more deeply and is completely enveloped in STB. Subsequently, primary villi originating from the cytoTB breach the STB to initiate formation of the villous placenta (not shown). Modified from Figure 12.17, page 397 in Developmental Biology/Scott F. Gilbert 11th Ed [2016] with permission.



Functionalis shed at each menstruation



(From Cunningham FG, MacDonald PC, Gant NF, et al. The endometrium and decidua: menstruation and pregnancy. In Williams Obstetrics, 19th ed. Stamford, CT: Appleton & Lange, 1993:81-105.)

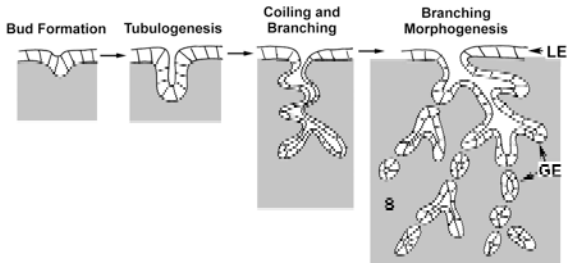
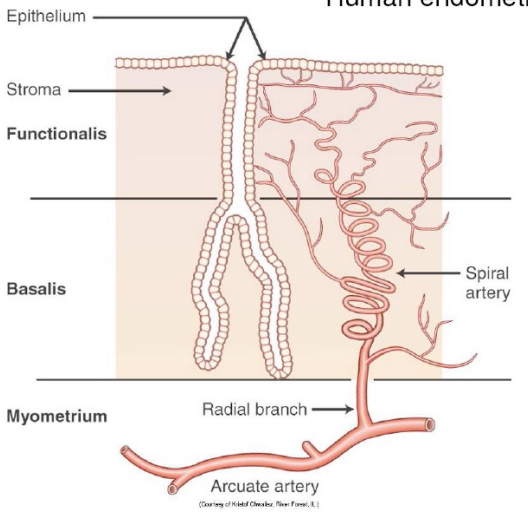


Figure 1. General illustration of the process of endometrial gland development. Uterine glands originate as shallow gland buds from the lumenal epithelium (LE) before undergoing invagination to form tubules. As the tubules progress through the stroma (S) toward the myometrium, they begin to coil and branch. The final stage of endometrial glandular epithelial (GE) differentiation is the process of branching morphogenesis, which does not occur in rodents. This process is similar to that occurring in epitheliomesenchymal organs, such as the lung, salivary gland, prostate, and mammary gland.

Human endometrium



(Courtesy of Harold Chrastil, River Forest, IL.)

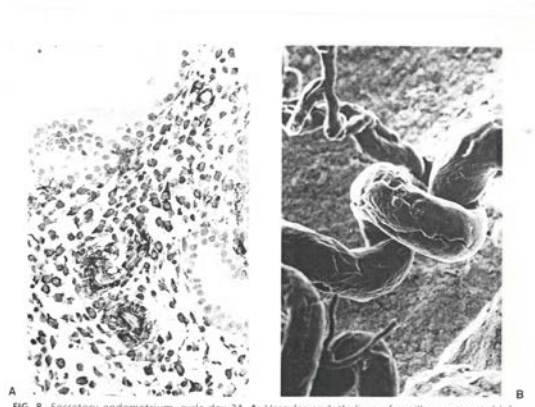


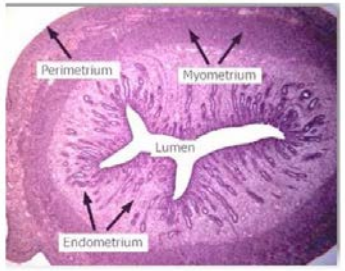
FIG. 8. Secretory endometrium, cycle day 24. A: Vascular endothelium of capillary system is highlighted by factor VIII antibody immunostaining (H&E ×350). B: Scanning electron microscopy of tortuous endometrial vessel with collateral branching *(lower right)* (×10,000).

Female Reproductive Anatomy

Uterus

Prominent organ of the female reproductive tract, organ of pregnancy

1. serosa – perimetrium
2. muscularis – myometrium
3. mucosa – endometrium



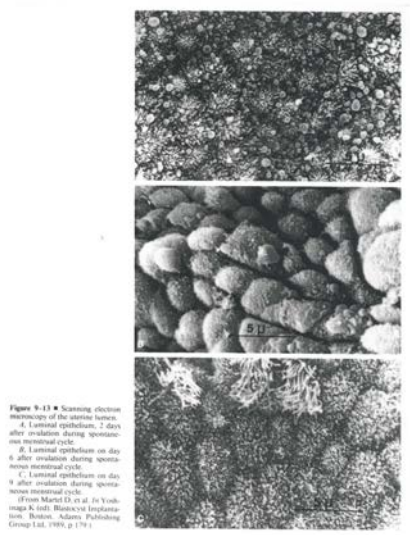
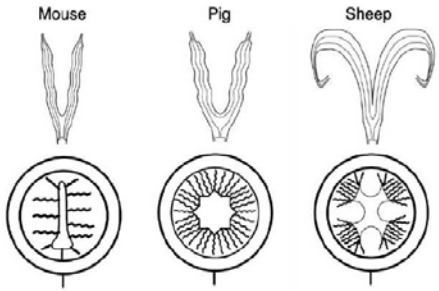


Figure 9-13 ■ Scanning electron microscopy of the uterine lumen. *A*, Luminal epithelium, 2 days after ovulation during spontaneous menstrual cycle. *B*, Luminal epithelium on day 6 after ovulation during spontaneous menstrual cycle. *C*, Luminal epithelium on day 9 after ovulation during spontaneous menstrual cycle. (From Martel D, et al: In Yoshimaga K (ed). Blastocyst Implantation. Boston, Adams Publishing Group Ltd, 1989, p 179.)



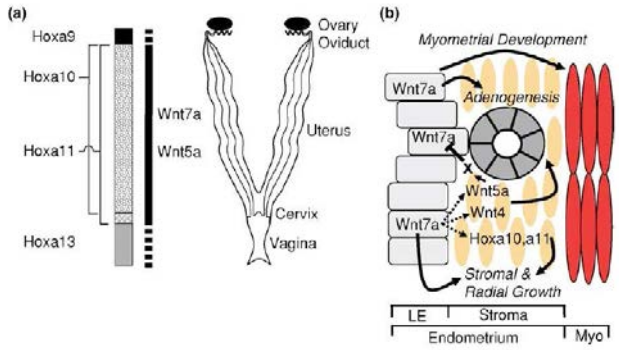
Curved lines - tubular, coiled, and branched glands that extend from the uterine lumen to the inner layer of myometrium

rodent uterus - a few endometrial glands

sheep uterus contains a large number of glands in the intercaruncular areas of the endometrium

pig uterus contains large numbers of glands throughout the endometrium

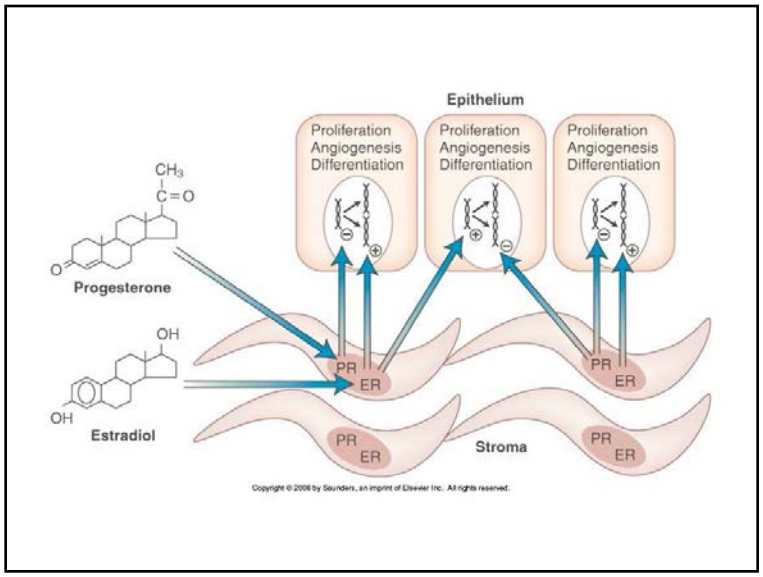
Spencer et al Curr Top Dev Biol 2005; 68:85



Female reproductive tract organogenesis in the mouse.

During fetal development – no region specific expression

Birth - Hoxa gene expression start to regionalize along the anteroposterior axis



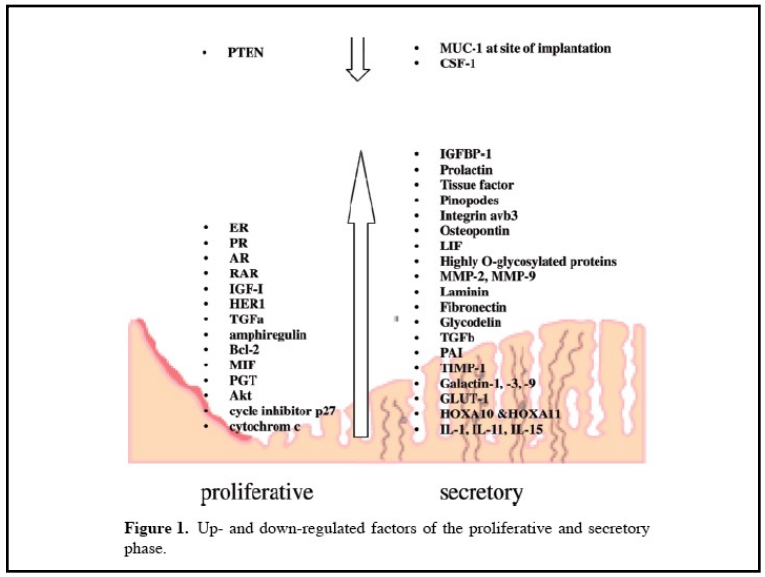
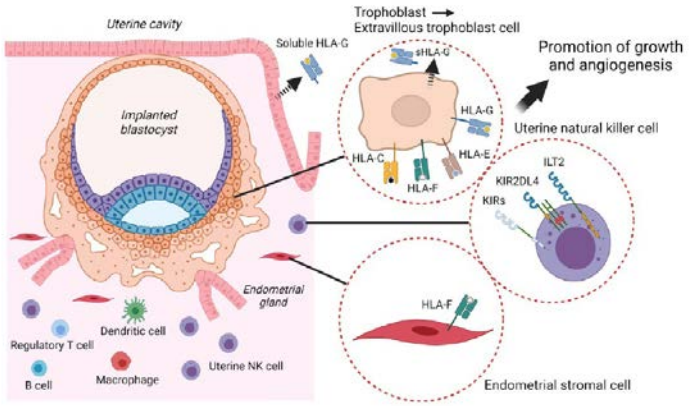
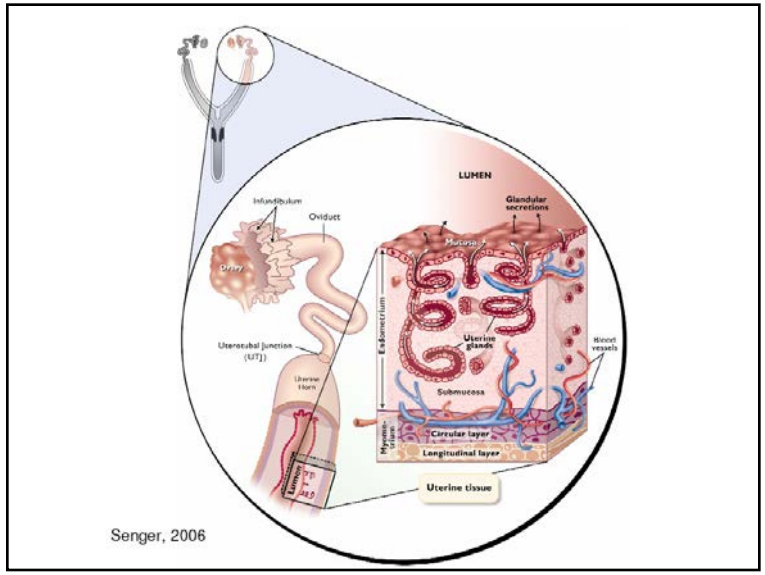


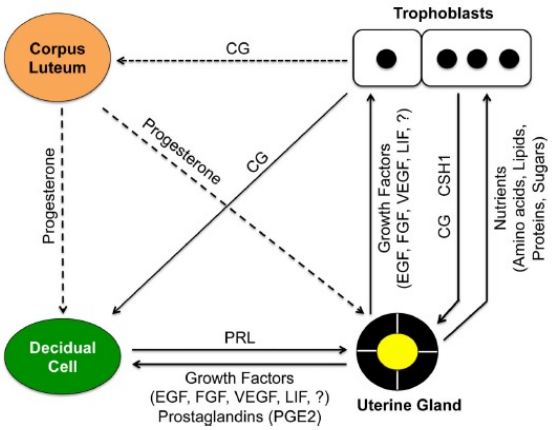
Figure 1. Up- and down-regulated factors of the proliferative and secretory phase.

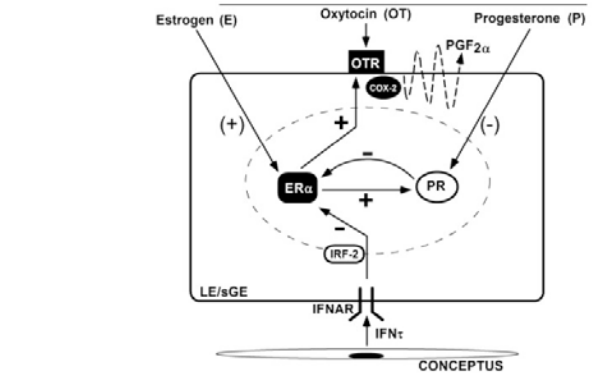
HLA Class Ib-receptor interactions during embryo implantation and early pregnancy.
Nilsson LL, Hviid TVF.
Hum Reprod Update. 2022 Mar 2;dmac007.





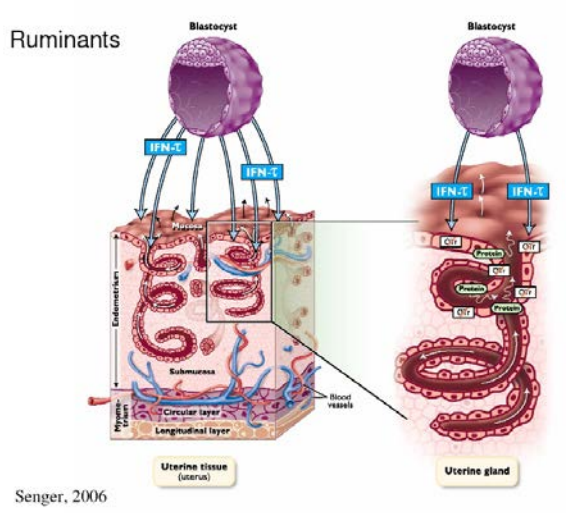
Senger, 2006



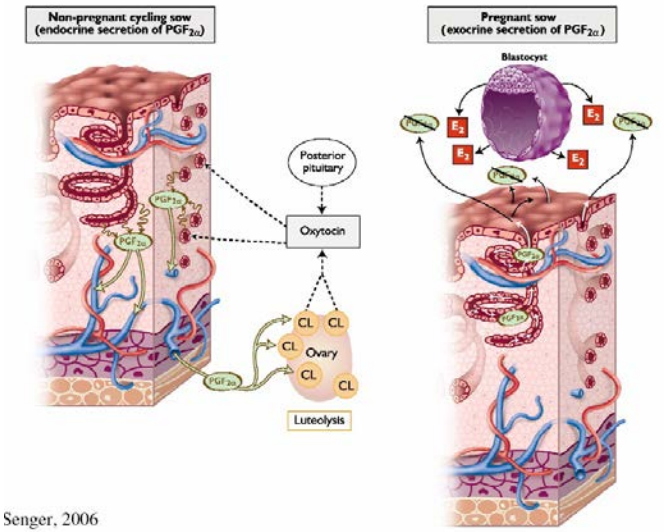


INFτ is critical day 15-16 bovine pregnancy, day 13-14 sheep

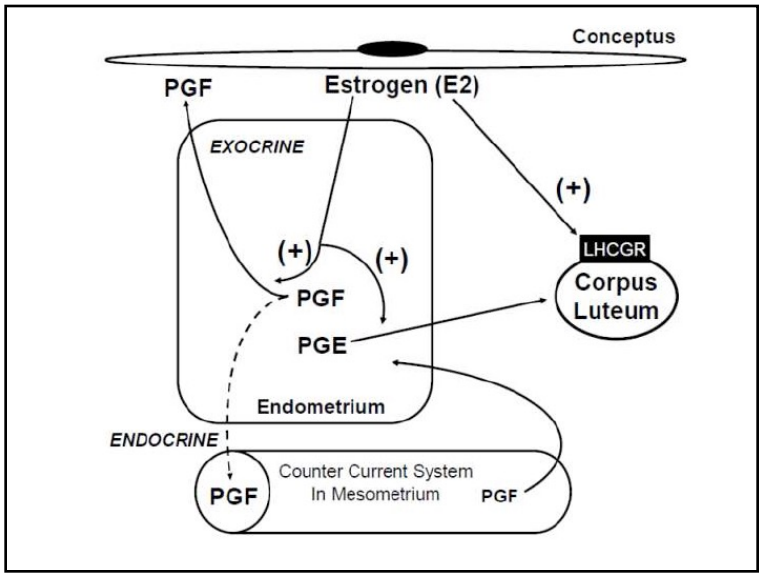
Spencer et al, BIOLOGY OF REPRODUCTION 71, 2–10 (2004)

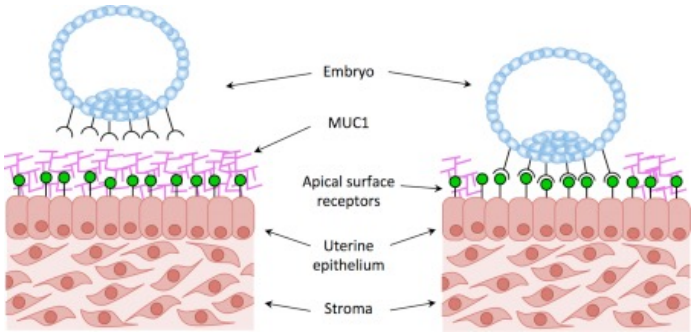


Senger, 2006



Senger, 2006





MUC1 as an anti-adhesion molecule acting as a barrier to embryo attachment to the apical surface of the uterine epithelium. MUC1 clearance allows access to the apical cell surface receptors for implantation.

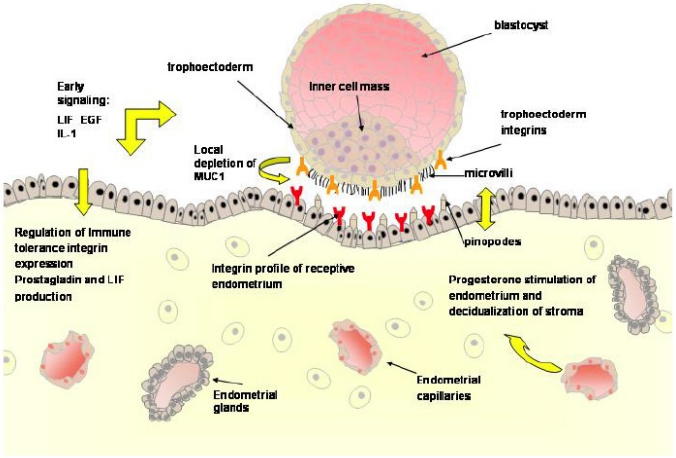


Figure 1
A schematic representation of a blastocyst approaching the receptive endometrium, defined by the integrin profile and appearance of pinopodes. Early signaling between the blastocyst and the endometrium precedes the attachment.

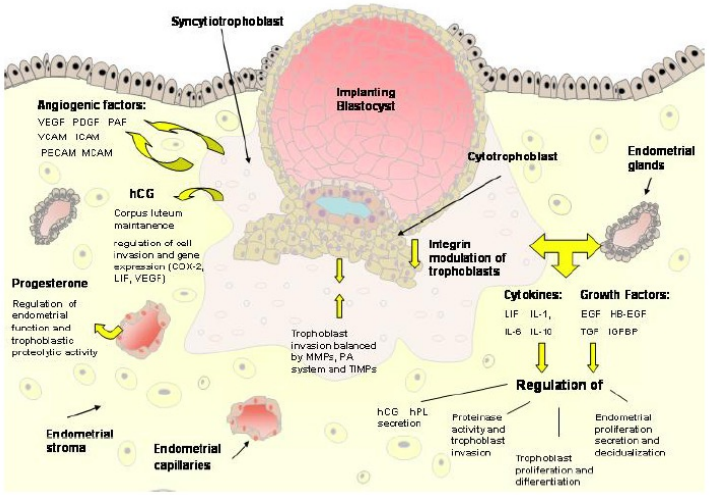
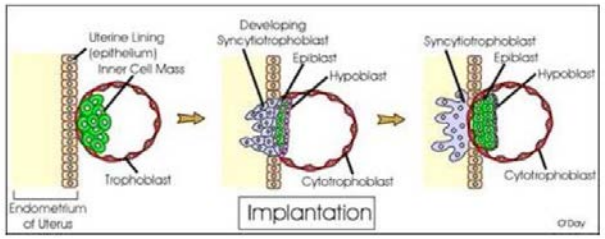


Figure 2
A schematic representation of an implanting blastocyst, highlighting interactions between trophoblastic and endometrial cells, including integrins, growth factors, cytokines, hormones and proteases.



Apposition

Adherence

Formation of Syncytiotrophoblast: Fusion of cytotrophoblast cells results in giant multinucleate cell that will surround complete embryo

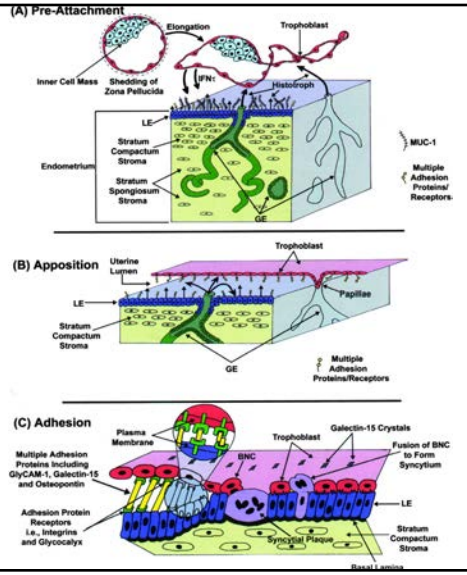
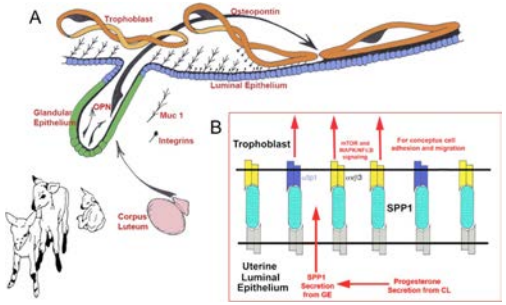


Figure 2 Apposition and adhesion phases of blastocyst implantation in sheep. (A) Preattachment involving shedding of the zona pellucida (phase 1) and precontact and blastocyst orientation (phase 2). The first phase of implantation is the shedding of the zona pellucida on day 8 that exposes the trophoblast. The second phase of implantation is before contact and involves the blastocyst orientation which occurs on days 9–11 as the blastocyst grows from a spherical to a tubular conceptus and migrates to the middle region of the uterine horn ipsilateral to the corpus luteum. The antiadhesive mucin MUC-1 is present on the endometrial LE, thereby preventing contact of the trophoblast with adhesive receptors such as integrins. Histotroph, secreted from the endometrial LE and GE, nourishes the developing blastocyst. (B) Apposition and transient attachment (phase 3). After day 11, the tubular blastocyst elongates to form a filamentous conceptus. During this period, expression of MUC-1 declines on the LE, which exposes constitutively expressed integrins on the LE as well as trophoblast. Apposition occurs between the trophoblast and endometrial LE and between the trophoblast papillae and GE ducts. Elongation of the blastocyst probably requires apposition and transient attachment to the endometrial LE. (C) Adhesion (phase 4). Firm adhesion of the mononuclear cells of the trophoblast to the LE occurs between days 15 and 16. Available evidence indicates that several molecules (GlyCAM-1, galectin-15 and osteopontin) interact with receptors (integrins and glycoconjugates) on the apical surfaces of the trophoblast and LE to facilitate adhesion. The binucleate cells (BNC) differentiate from the mononuclear trophoblast and then migrate to and fuse with the LE to form a multinucleated syncytial plaque. Although the BNC are inherently invasive, they do not cross the basal lamina that supports the LE.

Osteopontin: a leading candidate adhesion molecule for implantation in pigs and sheep.

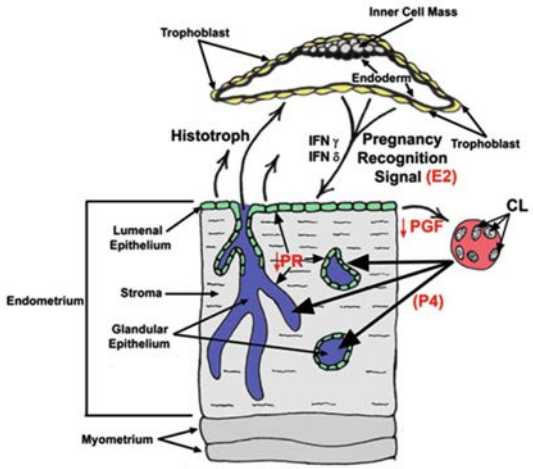
Johnson GA, Burghardt RC, Bazer FW.
J Anim Sci Biotechnol. 2014 Dec 17;5(1):56.

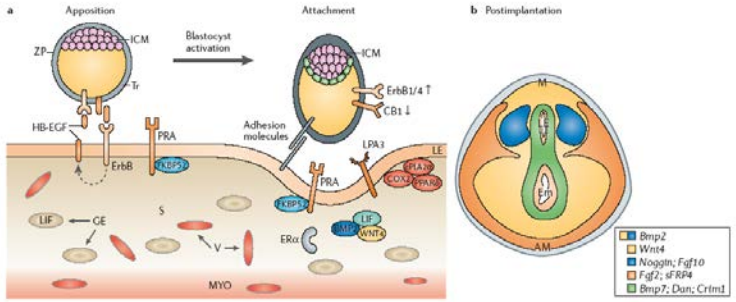


Expression, regulation and proposed function of OPN produced by the uterine GE of pregnant sheep. A) As the lifespan of the CL is extended as the result of the actions of interferon tau secretion from elongating ovine conceptuses (Trophoblast) they secrete progesterone. Progesterone then induces the synthesis and secretion of OPN (Osteopontin) from the uterine GE (Glandular Epithelium)[51]. The implantation cascade is initiated with down-regulation Muc 1 (the regulatory mechanism remains to be identified) on the LE surface to expose integrins on the LE and trophoblast surfaces for interaction with OPN to mediate adhesion of trophoblast to LE for implantation[29, 51, 52, 66]. B) In vitro experiments have identified the αvβ3 integrin receptor on trophoblast as a binding partner for OPN[66]. OPN then likely acts as a bridging ligand between αvβ3 on trophoblast and as yet unidentified integrin receptor(s) expressed on the opposing uterine LE. Note that the α5 integrin subunit was immunoprecipitated from membrane extracts of biotinylated oTr1 cells that were eluted from an OPN-Sepharose column, but the β1 integrin subunit, the only known binding partner for α5, could not be immunoprecipitated. Therefore, while we cannot definitively state that OPN binds α5β1 integrin on oTr1, we are reticent to exclude this possibility.

Insights into the Regulation of Implantation and Placentation in Humans, Rodents, Sheep, and Pigs

Stenhouse C, Seo H, Wu G, et al.
Adv Exp Med Biol. 2022;1354:25-48.





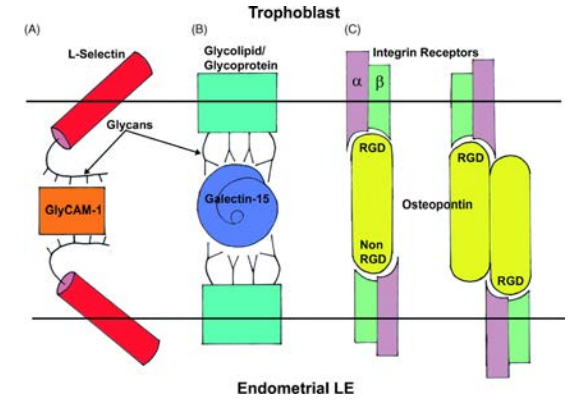
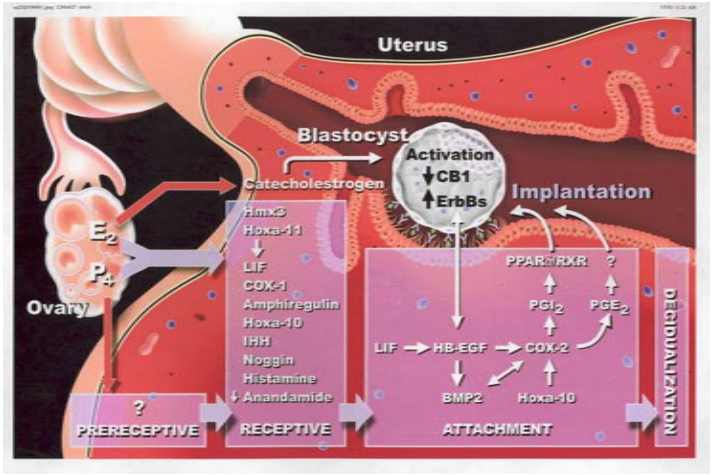


Figure 3 Possible interactions between trophoblast and endometrial epithelial cells regulating adhesion during implantation. Evidence for these interactions are from *in vivo* and *in vitro* studies in sheep, mice and humans. (A) Homotypic binding of GlyCAM-1 by L-selectin. GlyCAM-1 functions as a carbohydrate ligand for the lectin domain of L-selectin. (B) Homotypic and heterotypic binding of glycans on proteins and/or lipids via the multifunctional, secreted bridging ligand galectin-15. There is evidence that galectin-15 can bind to a large number of proteins and lipids, including mucins and integrins, which contain ß-galactosides, using a carbohydrate recognition domain. (C) Homotypic and heterotypic integrin-mediated adhesion via the bifunctional, secreted bridging ligand OPN. There is evidence that OPN can homodimerize and bind integrins in a RGD-dependent and RGD-independent manner.

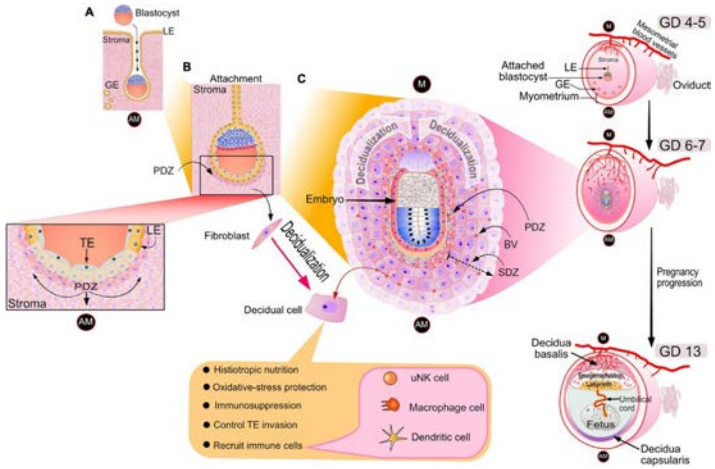
Genes expressed in the glandular epithelia of the mouse uterus and effects of mutation on pregnancy outcomes

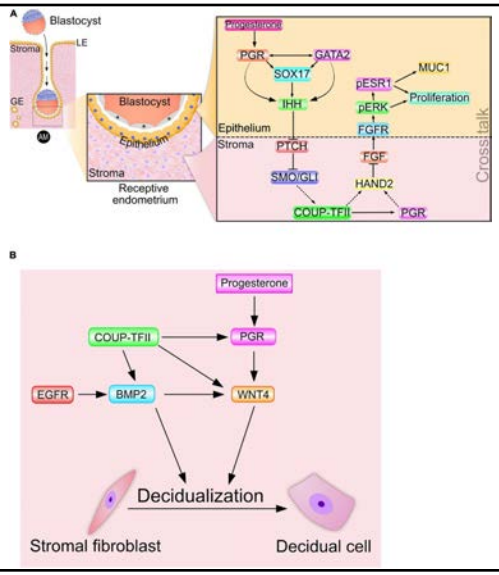
Symbol	Name	Expression[1]	Null/Conditional Phenotype	Reference
Cdh1	E-cadherin	LE and GE (GD1-4), stroma (GD5-8)	Embryonic lethal, Implantation defect (conditional)	101,102
Clca3	chloride channel calcium activated 3	LE and GE (peaks on GD1)	Viable and fertile	103,104
Cxcl15	chemokine (C-X-C motif) ligand 15	GE, LE	Viable and fertile	105,106
Fmbp1	formin binding protein 1	GE (GD4-5)	N/A	44
Foxa2	forkhead box A2	GE (neonatal and adult)	Embryonic lethal, Implantation defect (conditional)	35,107
Gulo	gulonolactone (L-) oxidase	GE>>LE (GD4-5)	Viable and fertile	44,108
Ihh	Indian Hedgehog	LE and GE (peaks on GD3-4)	Embryonic lethal, Implantation defect (conditional)	109
Il6st	interleukin 6 signal transducer	GE (GD3-5) Decidua (GD7)	Viable and fertile	110,111
Klf5	Kruppel-like factor 5	LE and GE (GD1-5) Decidua (GD5-8)	Embryonic lethal, Implantation defect (conditional)	112
Lgr4	leucine-rich repeat-containing G protein-coupled receptor 4	GE, LE	Implantation defect	41
Lif	leukemia inhibitory factor	GE>>Stroma (GD4)	Implantation defect	34
Ltf	lactotransferrin	LE and GE (GD1-2)	Viable and fertile	113,114
Lyz2	lysozyme 2	Stroma, GE (GD3-5)	Viable and fertile (knock-in)	44,115
Msx1	homeobox, msh-like 1	LE and GE (peaks on GD4 and declines)	Embryonic lethal, Subfertile (conditional)	116,117
Msx2	homeobox, msh-like 2	LE and GE (peaks on GD4)	Viable and fertile, Infertile (double conditional)	116,117
Prss28	protease, serine, 28	GE (GD5-8)	N/A	118
Prss29	protease, serine, 29	GE (GD5-9)	N/A	119
Ptgs1	Prostaglandin-endoperoxide synthase 1	LE and GE (peaks on GD4)	Viable, delayed parturition	120,121
Sh3tc2	SH3 domain and tetratricopeptide repeats 2	GE (GD4-5)	Viable and fertile	44,122
Slc23a2	solute carrier family 23 (nucleobase transporters), member 2	GE	Postnatal lethal	44,123
Spink3	serine peptidase inhibitor, Kazal type 3	GE (onset GD4)	Postnatal lethal	54,124
Sult1d1	sulfotransferase family 1D, member 1	GE>>LE (GD3-4)	N/A	44
Tro	Trophinin	LE and GE (peaks between GD4 and 6)	Viable and fertile	125
Ttr	transthyretin	GE only (peaks on GD4)	Viable and fertile	126,127

[1] GD, gestational day; GE, glandular epithelium; LE, luminal epithelium

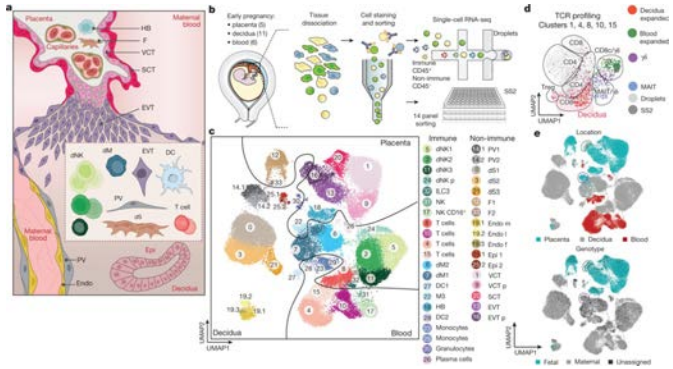


Illuminating the "Black Box" of Progesterone-Dependent Embryo Implantation Using Engineered Mice
Vineet K Maurya, Francesco J DeMayo, John P Lydon
Front Cell Dev Biol. 2021 Apr 7;9:640907.

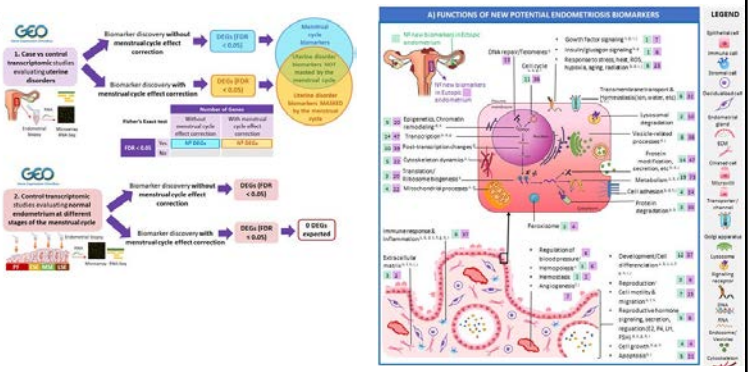




Single-cell reconstruction of the early maternal-fetal interface in humans.
Vento-Tormo R, Efremova M, Botting RA, et al.
Nature. 2018 Nov;563(7731):347-353.



Guidelines for biomarker discovery in endometrium: correcting for menstrual cycle bias reveals new genes associated with uterine disorders.
Devesa-Peiro A, Sebastian-Leon P, Pellicer A, Diaz-Gimeno P.
Mol Hum Reprod. 2021 Mar 24;27(4):gaab011.



"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