

**Spring 2020 – 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 14, 2020**  
**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.

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## Fertilization in the Oviduct

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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 as 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 epregrulin 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 epregrulin 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 seminal 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, PLC $\zeta$ , which plays a key role in (Clift and Schuh, 2013; Yeste et al., 2017). PLC $\zeta$ , 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 II $\gamma$  (CaMKII $\gamma$ ) (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<sub>3</sub> 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 PLC $\zeta$  (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.

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# FERTILIZATION

## Fertilization, Comparative

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### 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.

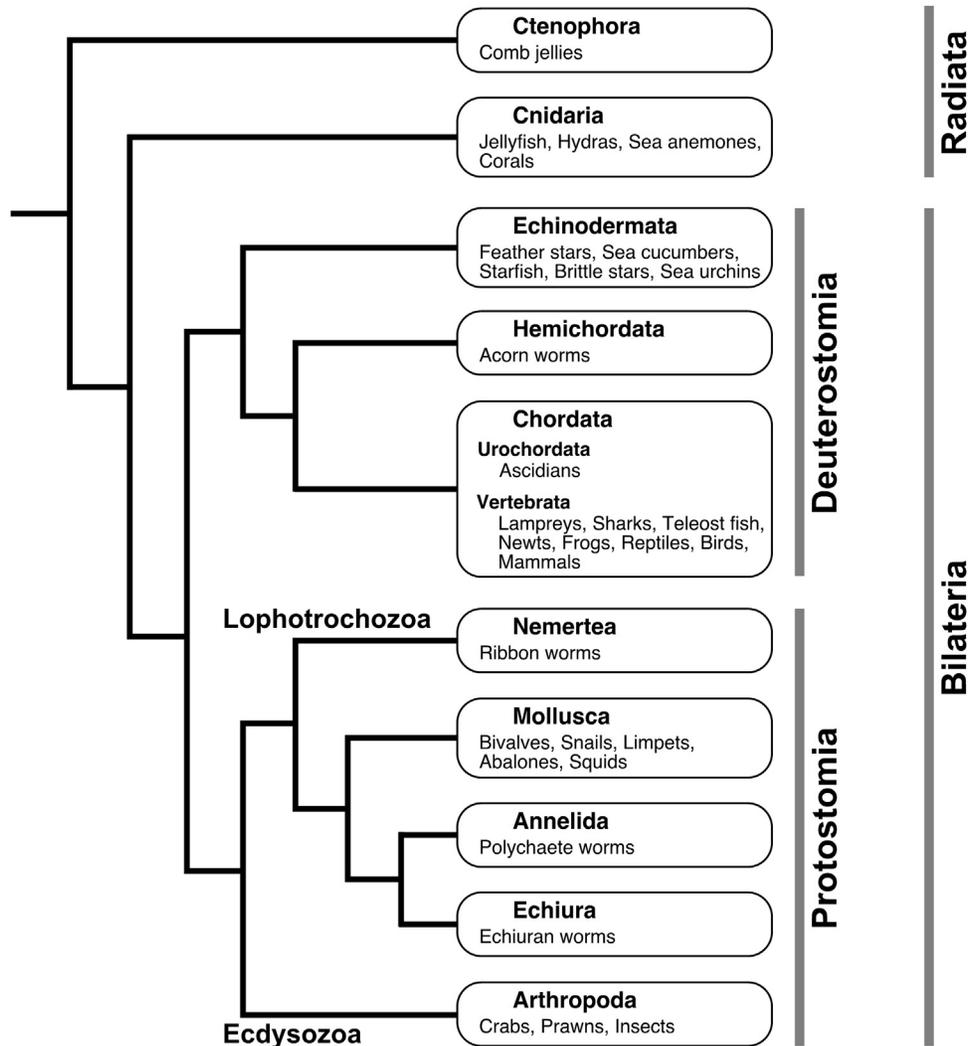
### The Site of Fertilization

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 portion 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  $\text{Ca}^{2+}$  and  $\text{Na}^{+}$  and the efflux of  $\text{H}^{+}$  and  $\text{K}^{+}$ . In particular,  $\text{Ca}^{2+}$  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, vesiculation 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 themselves 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  $\text{Ca}^{2+}$  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  $\mu\text{m}$  in diameter) can make a hole (3  $\mu\text{m}$  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  $\text{Na}^+$  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  $\text{Na}^+$ -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  $\text{Na}^+$  influx or  $\text{Na}^+$  and  $\text{Ca}^{2+}$  influxes is also thought to play a role in the fast polyspermy block. In contrast, in eggs of the crab *Maja squinado*, fertilization induces  $\text{K}^+$  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  $\text{Cl}^-$  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  $\mu\text{m}$  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

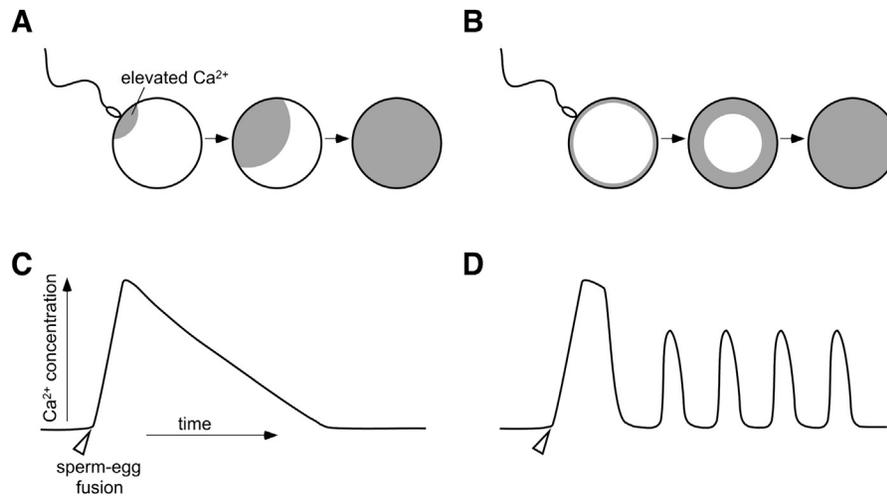
Fertilized eggs exhibit a series of processes required for the release from cell cycle arrest, by which normal development can start, which is collectively called egg activation. The key event that leads to egg activation is a transient rise in the cytoplasmic  $Ca^{2+}$  concentration in fertilized eggs (from 100 nM to 2–3  $\mu$ M 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  $\text{Ca}^{2+}$  rise in fertilized eggs. (A): Point-source  $\text{Ca}^{2+}$  wave initiated at the sperm-egg fusion site. (B): Centripetal  $\text{Ca}^{2+}$  wave initiated at the whole cortex. (C): Single  $\text{Ca}^{2+}$  rise. (D):  $\text{Ca}^{2+}$  oscillations.

polyspermy in the later phase.  $\text{Ca}^{2+}$  influx through voltage-dependent  $\text{Ca}^{2+}$  channels in many marine invertebrates and  $\text{Cl}^-$  efflux through  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  rise at fertilization also influence the later embryonic development (Whitaker, 2008). In ascidian and frog eggs, the direction of  $\text{Ca}^{2+}$  wave may specify the orientation of an embryonic axis. In mammals, abnormal patterns of  $\text{Ca}^{2+}$  oscillations are known to lower the developmental competence of blastocysts, resulting in fewer offspring.

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# Aspects of Rodent Implantation

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## 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 pre-implantation 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 trophoctoderm 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 trophoctoderm. 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 trophoctoderm 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<sub>4</sub> 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<sub>4</sub> 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<sub>4</sub>; surprisingly, neither P<sub>4</sub> 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<sub>4</sub> and estrogen secretion. To create the window of uterine receptivity, the cooperative interactions between P<sub>4</sub> 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, tamar wallaby and North American mink (Cha et al., 2013b). The molecular aspects of embryo competency and uterine receptivity are described in separate chapters.

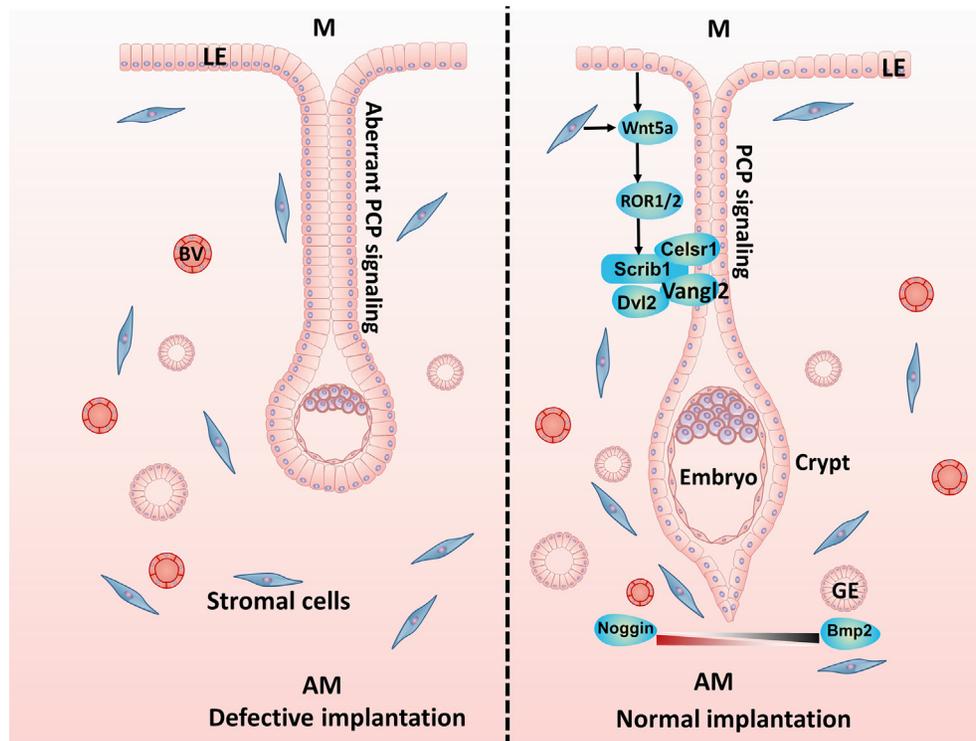
Blood vessels travel to the uterus through the mesometrium and anatomically orient the uterus into mesometrial and anti-mesometrial domains (Cha et al., 2012). In rodents, embryo homing and implantation occur within a crypt (implantation

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 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 cPLA $2\alpha$ ), 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.

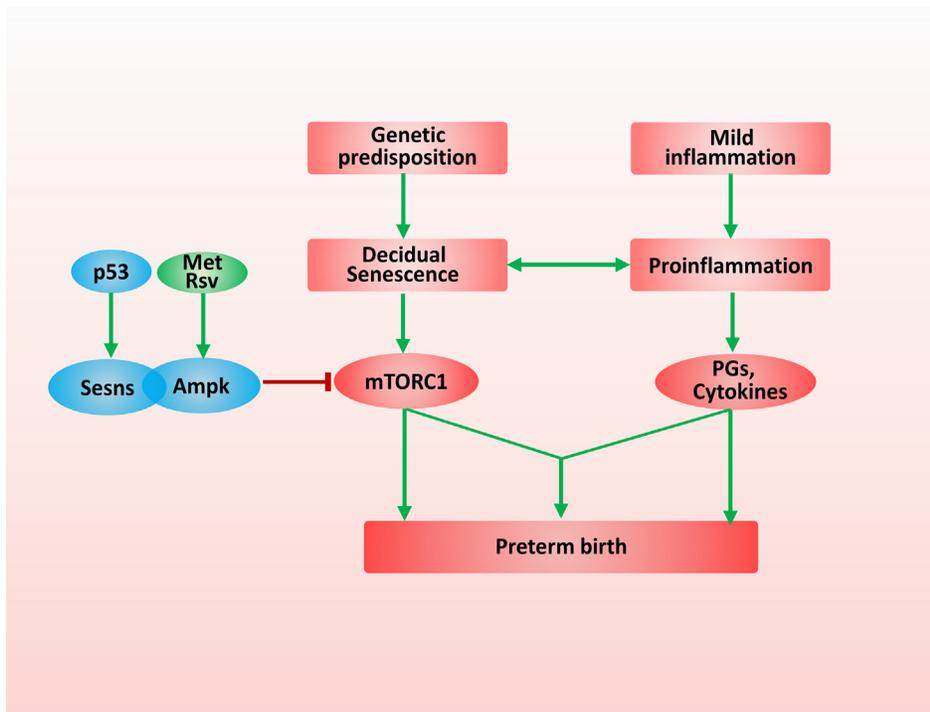
Preeclampsia is believed to result from abnormal placentation. A recent study in a mouse model which spontaneously develops the cardinal features of preeclampsia showed periimplantation defects including upregulation of Cox2 and IL-15 at the maternal-fetal interface (Sones et al., 2016). This was associated 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*<sup>d/d</sup>) show normal implantation, however, 50–60% of *Trp53*<sup>d/d</sup> 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*<sup>d/d</sup> decidia 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 decidua. 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.

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## Uterine Receptivity: The Status of Uterus for Implantation

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### 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

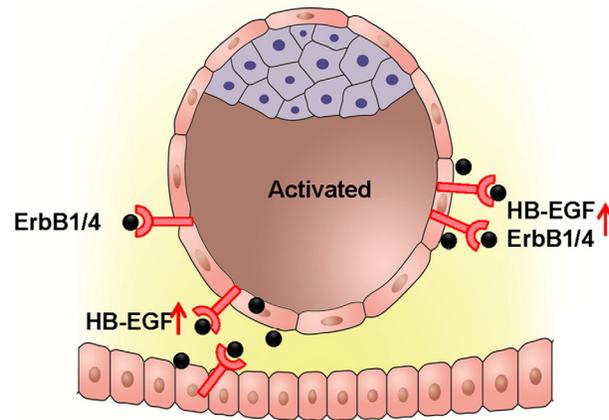
Luminal closure, defined as the closure of uterine lumen during embryo apposition prior to attachment, is another morphological landmark for uterine receptivity. This event supports a closer contact between the luminal epithelium and the blastocysts, which is essential for appropriate blastocyst apposition 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<sup>+</sup> 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 trophoblast in periimplantation blastocysts (Chobotova et al., 2002), indicating that HB-EGF/ErbB4 signaling also mediates the trophoblast–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 17 $\beta$ -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 ER $\alpha$  and ER $\beta$  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 ER $\alpha$ ) deletion is hypoplastic and unable to support implantation, whereas the uterus deficient for *Esr2* (gene encodes ER $\beta$ ) retains biological functions that allow for normal implantation, indicating the essential role of ER $\alpha$  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 trophoblast 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 ER $\alpha$ to stimulate the proliferation of uterine epithelium via paracrine factors

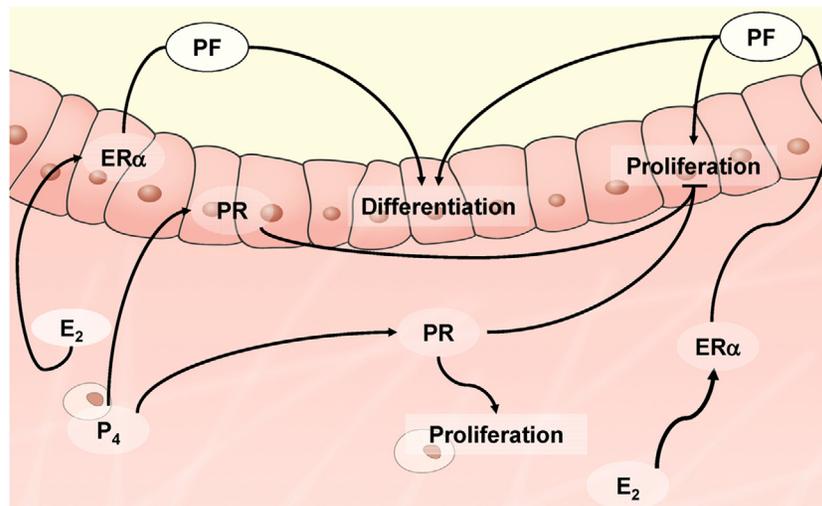
ER is expressed in both epithelial and stromal cells of the adult uteri. It was initially assumed that estrogen acts directly through the ER in the corresponding compartments. However, evidences from the experiments employing ESR1 knockout mouse models and stromal–epithelial separation/recombination systems (Cunha, 2008) demonstrated that estrogen could not stimulate epithelial proliferation in genetically recombined uterine tissue that lacks stromal ER $\alpha$ , even in the presence of epithelial ER $\alpha$ . The tissue-specific knockout techniques render the selective deletion of ESR1 in the uterine epithelium and further proved that stromal ER $\alpha$  is responsible for estrogen-induced epithelial proliferation. As to how the estrogen–ER $\alpha$  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  $\alpha$ . 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 ER $\alpha$  is also sufficient for estrogen-induced downregulation of PR in the uterine epithelial cells.

#### Differentiation of uterine epithelial cells requires both epithelial and stromal ER $\alpha$

Although it is the uterine stromal ER $\alpha$  not the epithelial ER $\alpha$  that is indispensable for estrogen-induced epithelial proliferation, the epithelial ER $\alpha$  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 ER $\alpha$  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).

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Spring 2020 – 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 14, 2020  
 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.

Fertilization

Spring 2020 – 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 16, 2020  
 Week 14

**Fertilization & Implantation Systems**

**Primary Papers:**

1. Teperek, et al. (2016) Genome Research 26:1034.
2. Brosens, et al. (2014) Scientific Reports 4:3894.
3. Vento-Tormo, et al. (2018) Nature 563(7731):347-353.

**Discussion**

Student 9: Reference 1 above

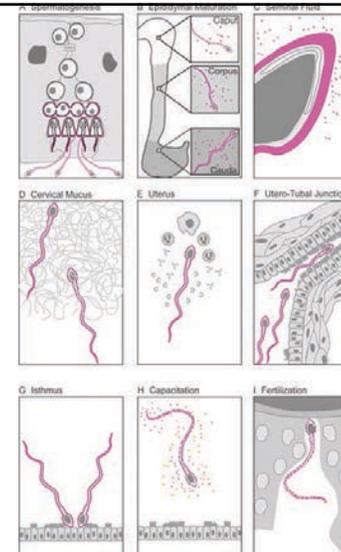
- What was the experimental design and objectives?
- What impact on the developing embryo was observed?
- Can sperm epigenetic alterations modify the embryo?

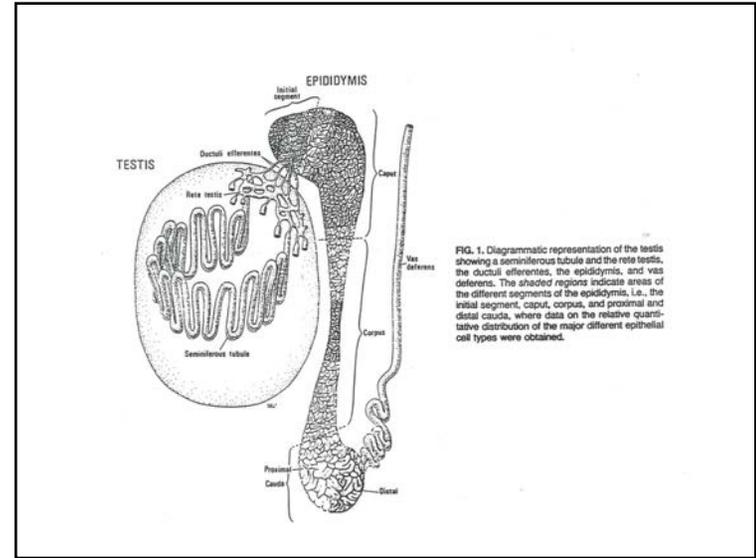
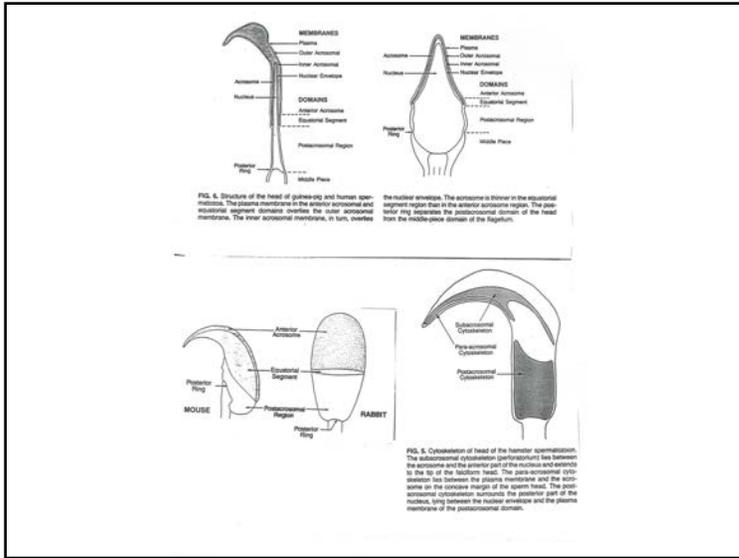
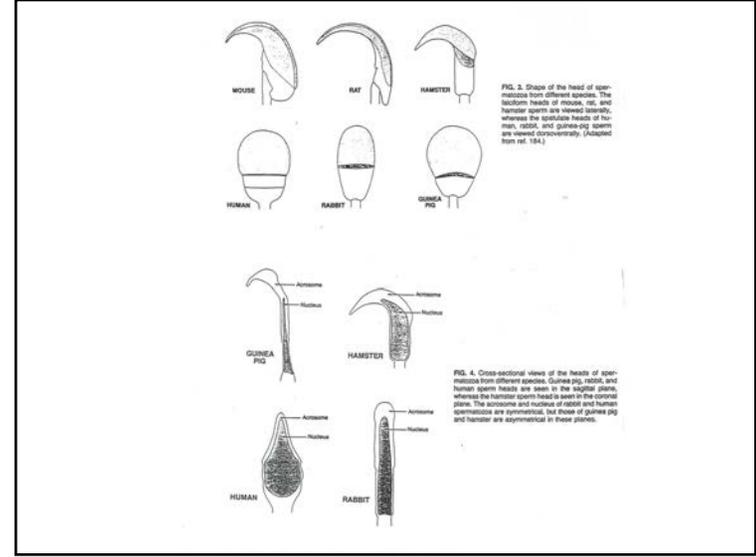
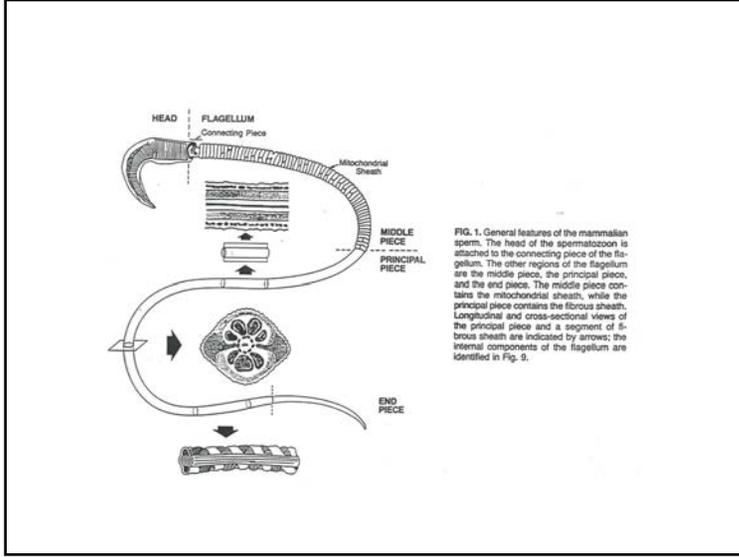
Student 10: Reference 2 above

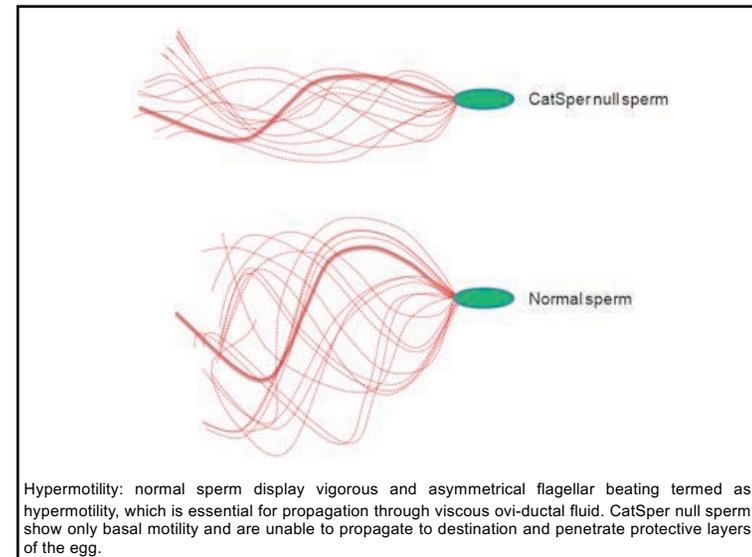
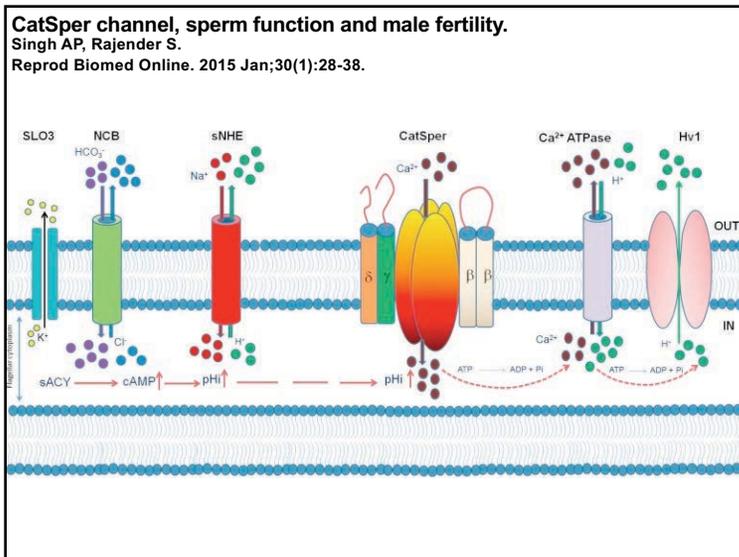
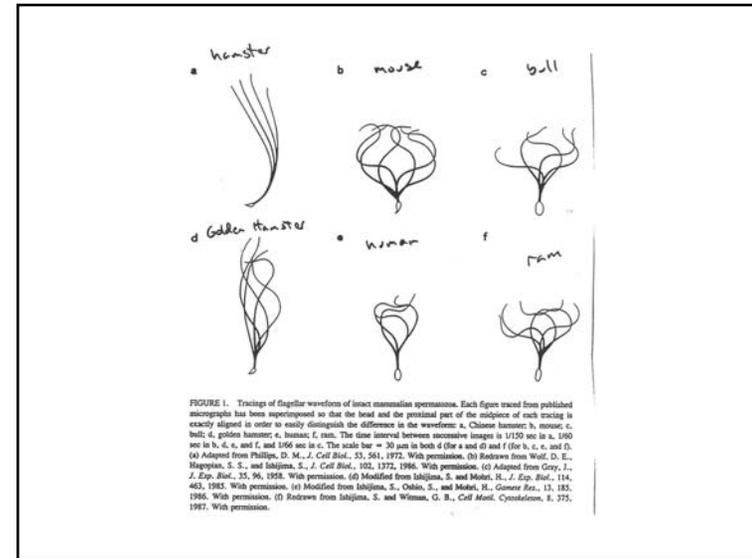
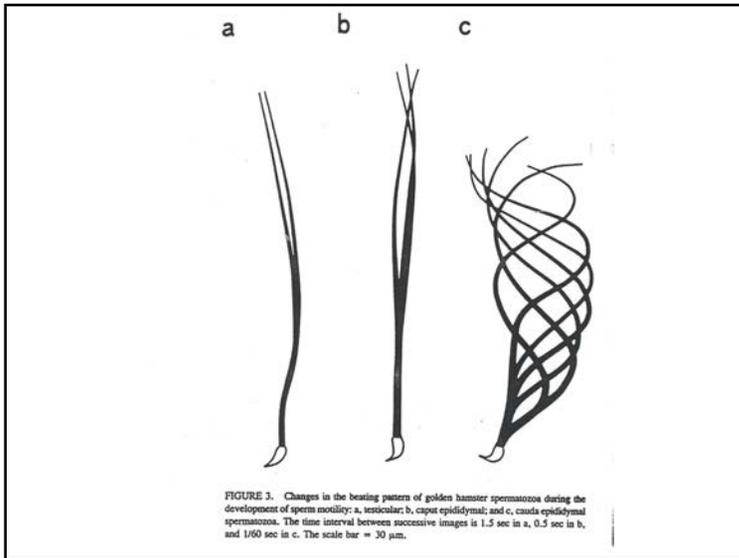
- How do abnormal embryos influence the endometrial cells?
- What transcriptional influences were observed?
- How do component embryos promote a supportive uterine environment?

Student 11: 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 placental and reproductive success?



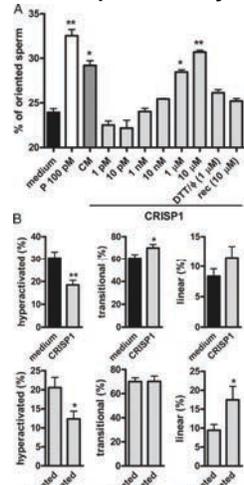




### CRISP1 as a novel CatSper regulator that modulates sperm motility and orientation during fertilization.

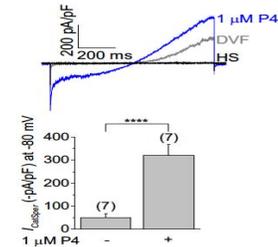
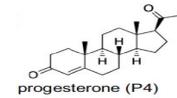
Ernesto JI, Weigel Muñoz M, Battistone MA, et al.  
*J Cell Biol.* 2015 Sep 28;210(7):1213-24.

Sperm orientation and motility in the presence of CRISP1.  
 (A) Capacitated sperm were placed in one well of a modified Zigmund chamber and CRISP1 (1 pM to 10 pM), DTT-treated and heat-denatured CRISP1 (1 pM; DTT/Φ), or recombinant CRISP1 (10 pM; 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 pM) 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.



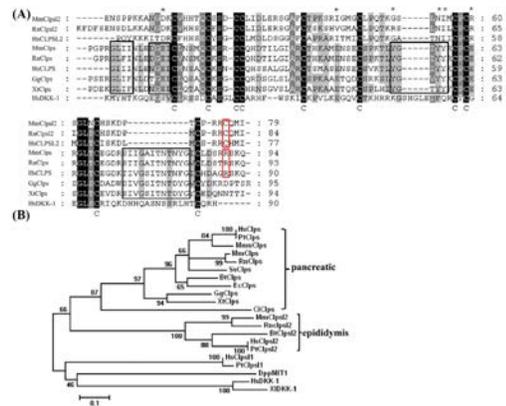
### Regulation of the sperm calcium channel CatSper by endogenous steroids and plant triterpenoids.

*Proc Natl Acad Sci U S A.* 2017 May 30;114(22):5743-5748.  
 Mannowetz N, Miller MR, Lishko PV.

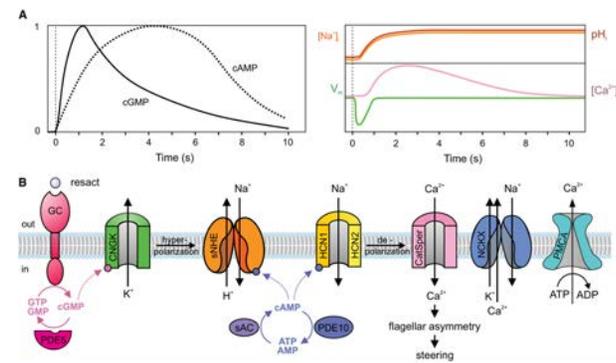


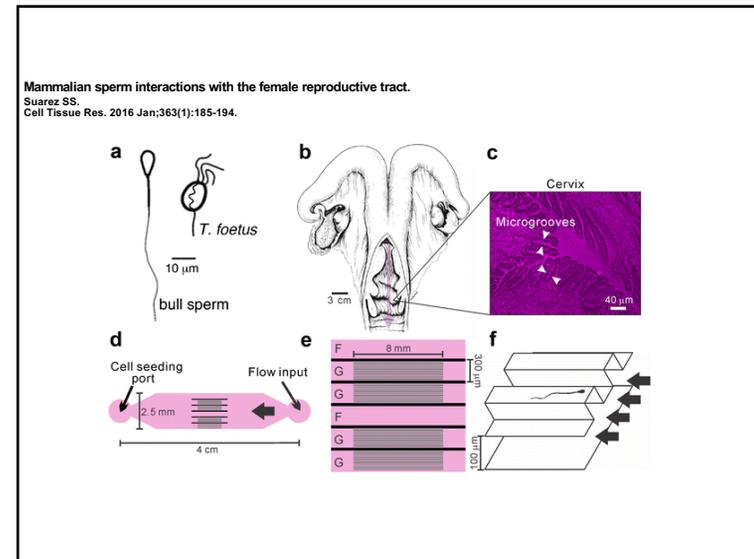
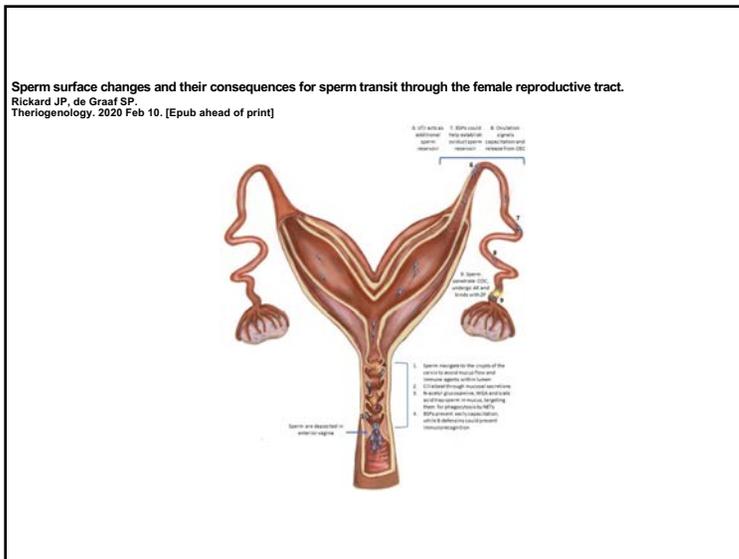
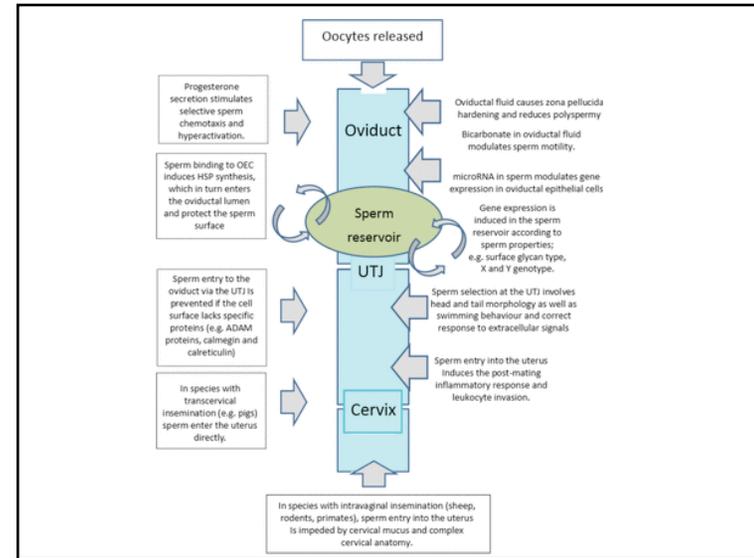
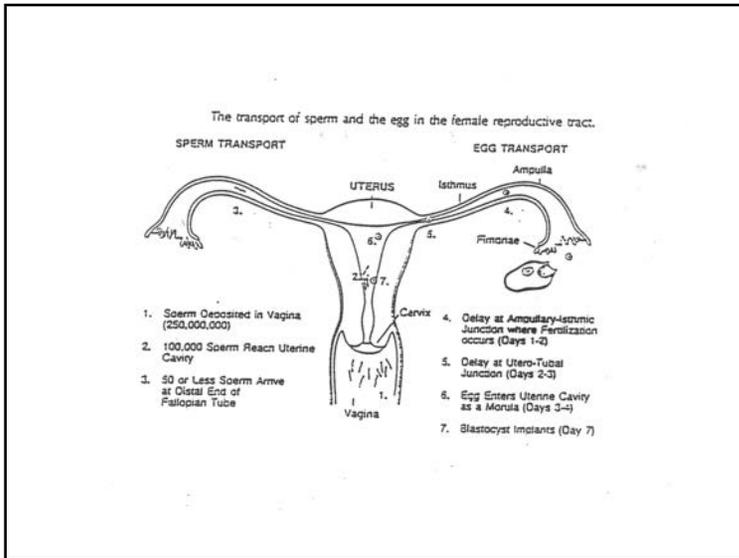
### An epididymis-specific secretory protein Clpsl2 critically regulates sperm motility, acrosomal integrity, and male fertility.

*J Cell Biochem.* 2018 Jan 11. doi: 10.1002/jcb.26668. [Epub ahead of print]  
 Lu X, Ding F, Lian Z, Chen L, Cao Z, Guan Y, Chen R, Cai D, Yu Y.



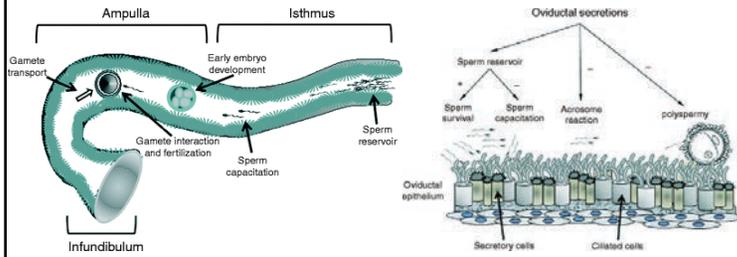
Absolute proteomic quantification reveals design principles of sperm flagellar chemosensation.  
 Trötschel C, Hamzesh H, Alvarez L, et al.  
*EMBO J.* 2020 Feb 17;39(4):e102723.



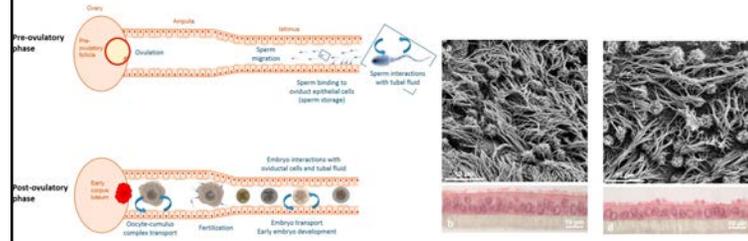


**Oviductal secretion and gamete interaction.**

Ghersevich S, Massa E, Zumoffen C.  
 Reproduction. 2015 Jan;149(1):R1-R14.

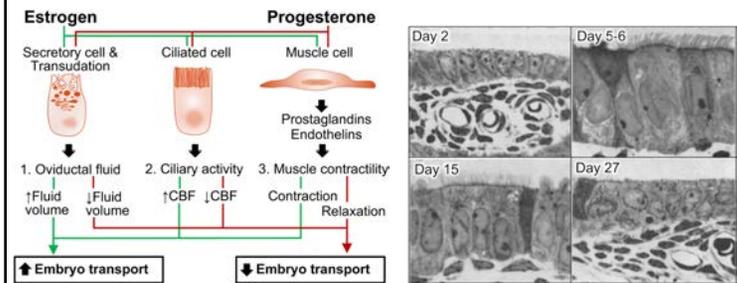


**Composing the Early Embryonic Microenvironment: Physiology and Regulation of Oviductal Secretions.**  
 Saint-Dizier M, Schoen J, Chen S, Banliat C, Mermillod P.  
 Int J Mol Sci. 2019 Dec 28;21(1).



**Roles of steroid hormones in oviductal function**

Barton BE, Herrera GG, Ananthakumala P, Rock JK, Willie A, Harris EA, Takemaru KI, Winuthayanon W.  
 Reproduction. 2020 Mar 1;159(3):R125-R137.



**a b Calcium reduced in sperm c De novo protein production by OEC d Progesterone increase at ovulation**

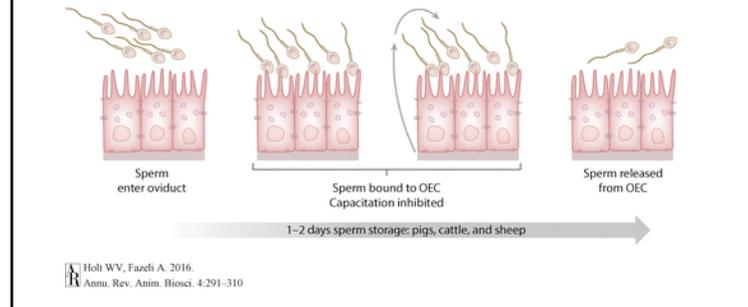
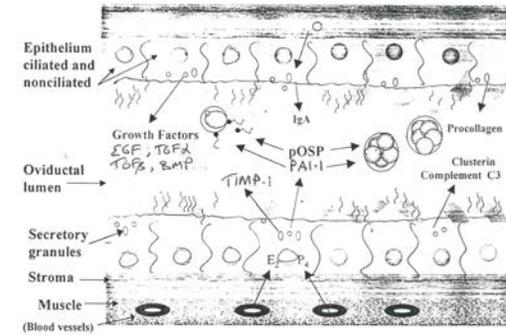
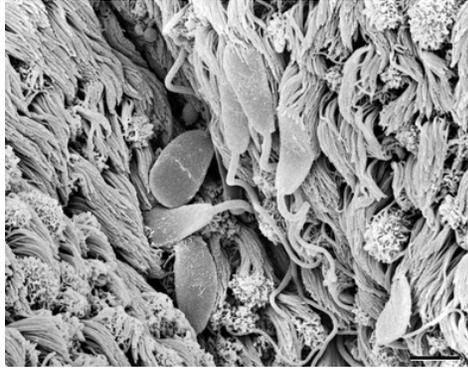
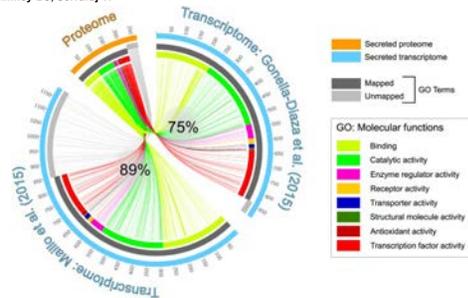


Diagram summarizing the main events that occur during oviductal sperm storage in pigs, cattle, and sheep. (a) Spermatozoa enter the oviductal isthmus via the utero-tubal junction. (b) Some spermatozoa bind to oviductal epithelial cell (OEC) surfaces, whereupon their motility and intracellular calcium 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).

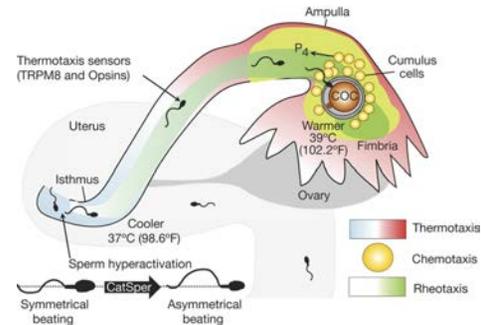


**Fig. 9.** Ciliated and secretory epithelium achieve maximum development, biosynthetic capacity and secretory activity during follicular development, ovulation and early cleavage-stage development. Secretory products are contributed as transudate and from active biosynthesis by secretory epithelium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

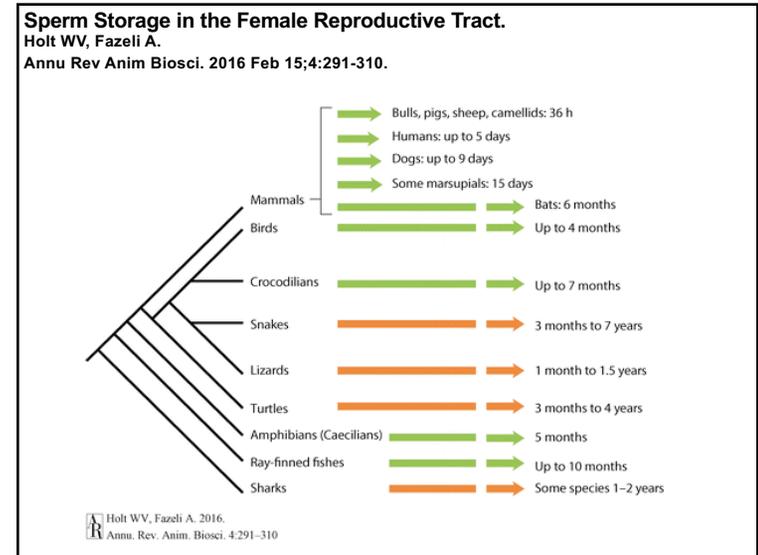
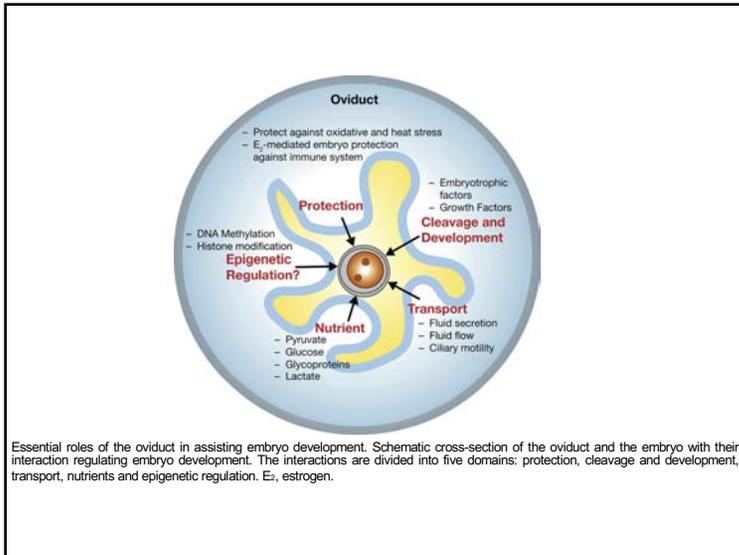
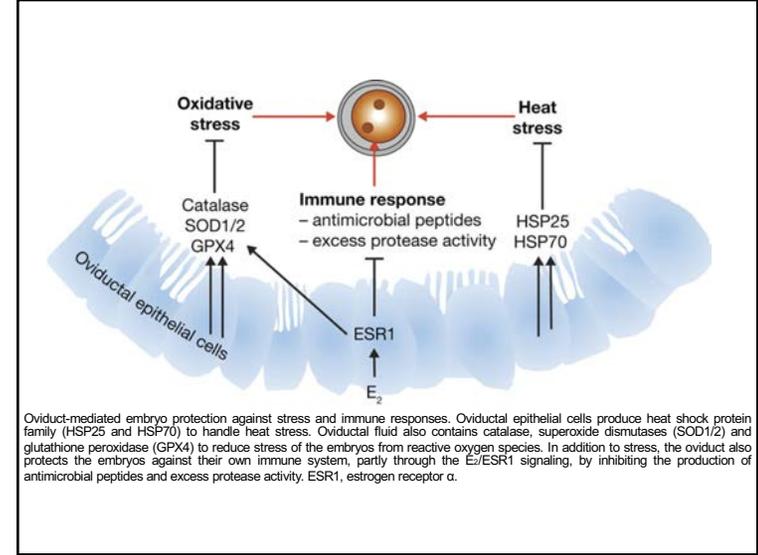
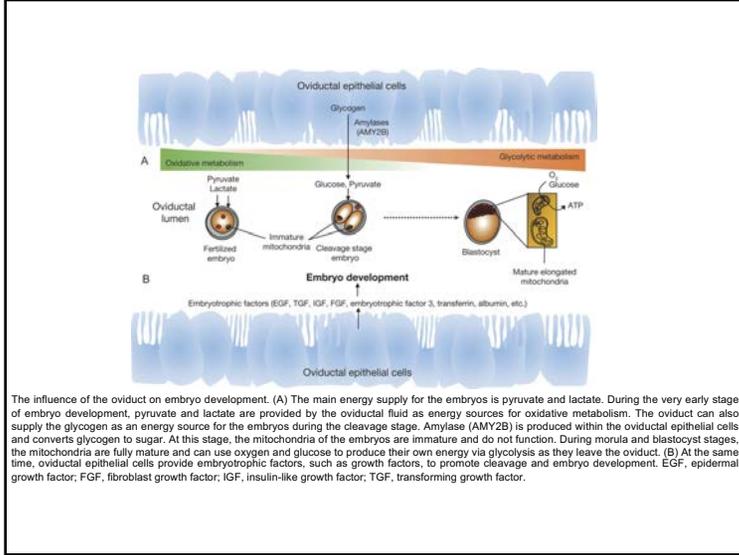
**Profiling of proteins secreted in the bovine oviduct reveals diverse functions of this luminal microenvironment.**  
 PLoS One. 2017, 12(11):e0188105.  
 Pillai VV, Weber DM, Phinney BS, Selvaraj V.



**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-Diaz 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 GatSper (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  $0.006^{\circ}C$  (Bahat et al. 2012). Temperatures depicted ( $37^{\circ}C$  vs  $39^{\circ}C$ ) 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.



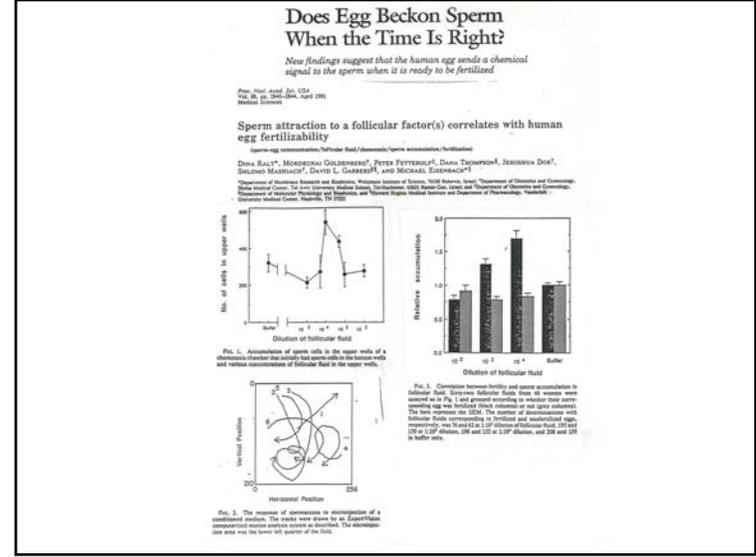
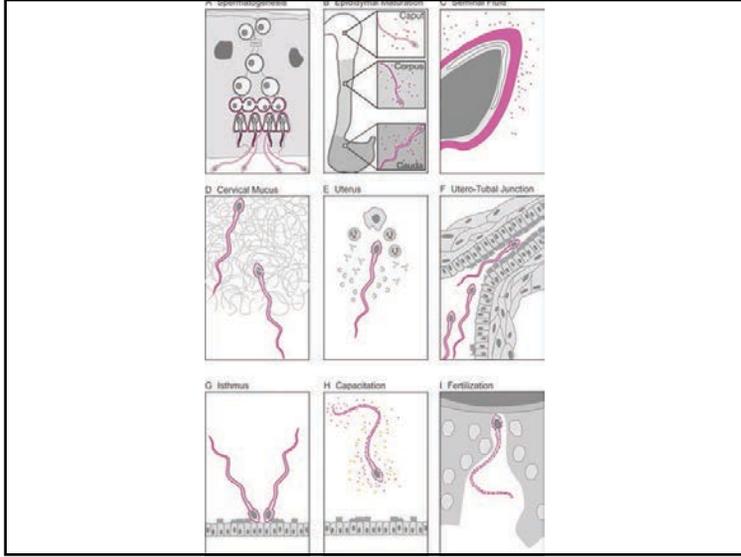
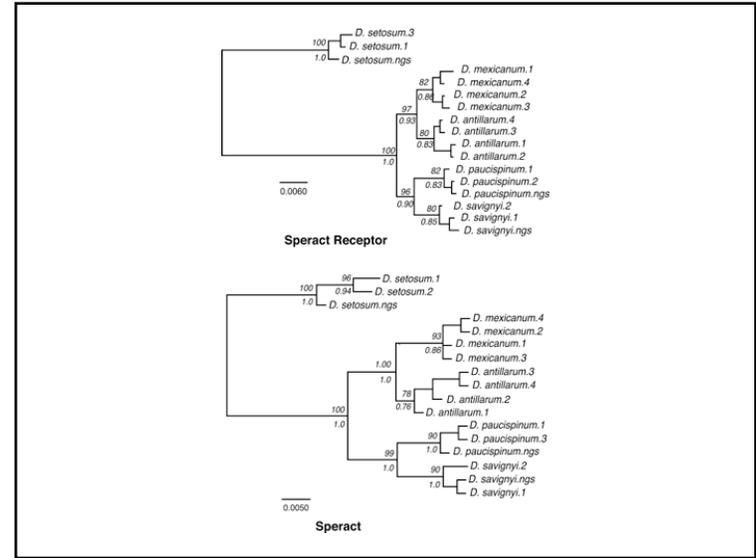


TABLE 1  
SUMMARY OF CHARACTERIZED CHEMOKINETIC AND CHEMOTACTIC MOLECULES AND THEIR EFFECTS ON SPERM

Species	Name	Structure	Derivatives*	Actions
<i>Strongylocentrotus purpuratus</i> <i>Hemicentrotus pulcherrimus</i> (sea urchin)	Speract (SAP I) <sup>1,4</sup>	Gly-Phe-Asp-Leu-Asp-Gly-Gly-Cys-Val-Gly	36 <sup>1,4,5</sup>	tcAMP, tcGMP [Ca <sup>2+</sup> ] <sub>i</sub> , tpH <sub>i</sub> [Respiration] [Motility] Cofactor in ABR (†)
<i>Artibeus penicillatus</i> (sea urchin)	Resact (SAP II) <sup>1</sup>	Cys-Val-Thr-Gly-Ala-Pro-Gly-Cys-Val-Gly-Gly-Gly-Arg-Leu-NH <sub>2</sub>	1	tcAMP, tcGMP [Respiration] [Motility] [Guanylyl cyclase] Chemoattractant tcAMP, tcGMP
<i>Glyptocidaris crenularis</i> <i>Stomagnatus varicosus</i> (sea urchin)	SAP IIB <sup>4</sup>	Lys-Leu-Cys-Pro-Gly-Gly-Asp-Cys-Val	6	tcAMP, tcGMP
<i>Cypraster japonicus</i> (sand dollar)	Mosact (SAP III) <sup>2,6</sup>	Asp-Ser-Asp-Ser-Ala-Glu-Asp-Leu-Ile-Gly	9	tcAMP, tcGMP
<i>Diadema setosum</i> (sea urchin)	SAP IV <sup>2</sup>	Gly-Cys-Pro-Trp-Gly-Gly-Ala-Val-Cys	1	tcAMP, tcGMP [Respiration]
<i>Briaros aperticil</i> (heart urchin)	SAP V <sup>2</sup>	Gly-Cys-Glu-Gly-Leu-Phe-His-Gly-Met-Gly-Asp-Cys	1	tcAMP, tcGMP
<i>Montipora digitata</i> (hard coral)	Compound 1	CH <sub>2</sub> (CH <sub>2</sub> ) <sub>2</sub> C-C-C-C-CH <sub>2</sub> OH	1	Chemoattractant
	Compound 2	CH <sub>2</sub> -C(CH <sub>2</sub> ) <sub>2</sub> C-C-C-CH <sub>2</sub> OH	1	Sperm activator
	Compound 3 <sup>7</sup>	CH <sub>2</sub> -C-C-Cl(CH <sub>2</sub> ) <sub>2</sub> -C-C-C-CH <sub>2</sub> OH	1	?
<i>Limulus polyphemus</i> (horseshoe crab)	SME <sup>8</sup>	Unknown (M <sub>r</sub> = 500-2000)	1	Initiates motility
<i>Chapes pallasi</i> (Pacific herring)	SME <sup>9</sup>	Unknown (M <sub>r</sub> = 105,000)	1	Initiates motility



## 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.)

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

Lindsay A. Burnett, Serenity Boyles, Christopher Sponora, Allan L. Biebra and Douglas E. Chandler Corresponding 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.

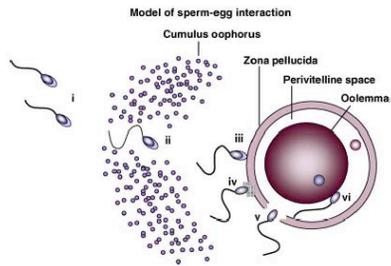
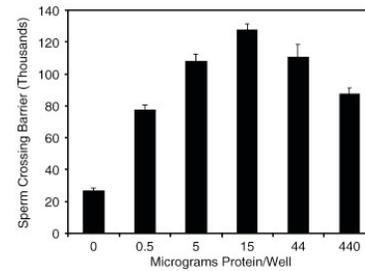


Fig. 1. Schematic diagram of the fertilization process. Within the female reproductive tract (i), sperm undergo a series of surface and intracellular transformations, collectively termed capacitation, which enables them to penetrate the cumulus oophorus (ii), bind to the zona pellucida (ZP) (iii), and 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).

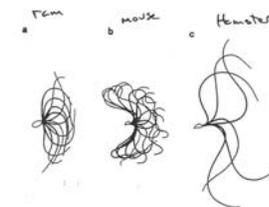
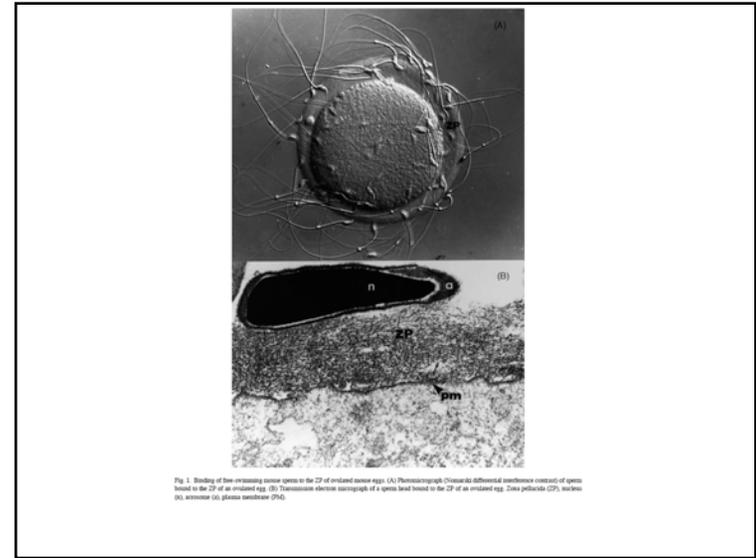
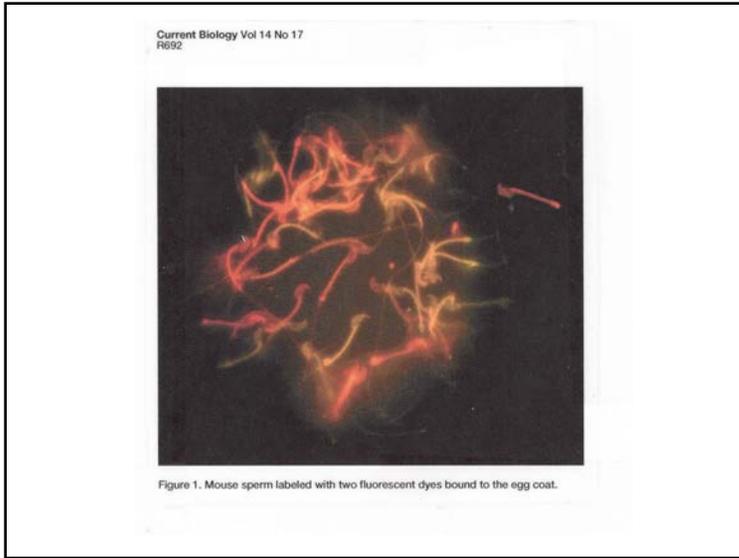
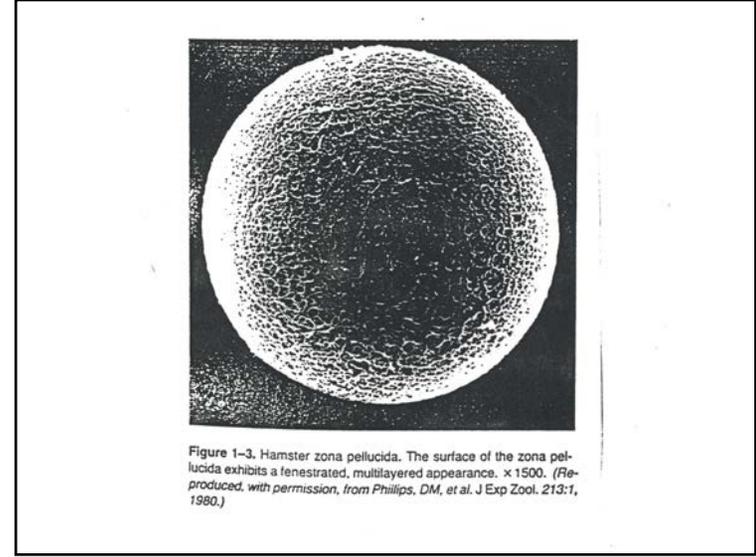
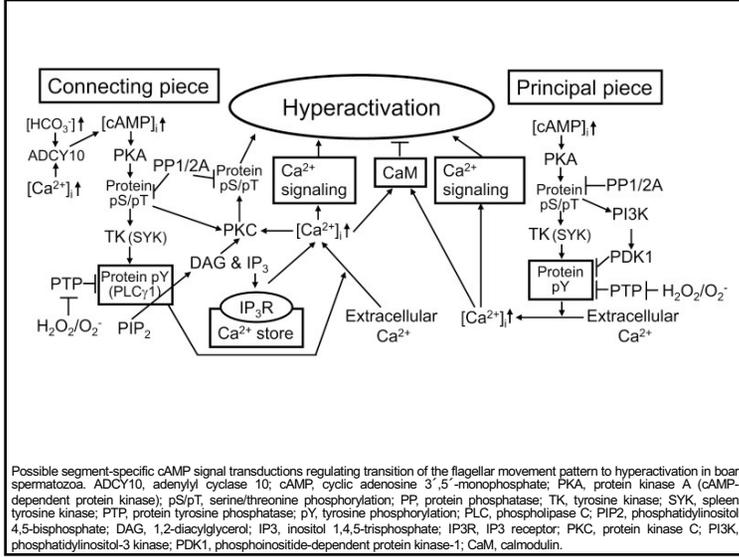


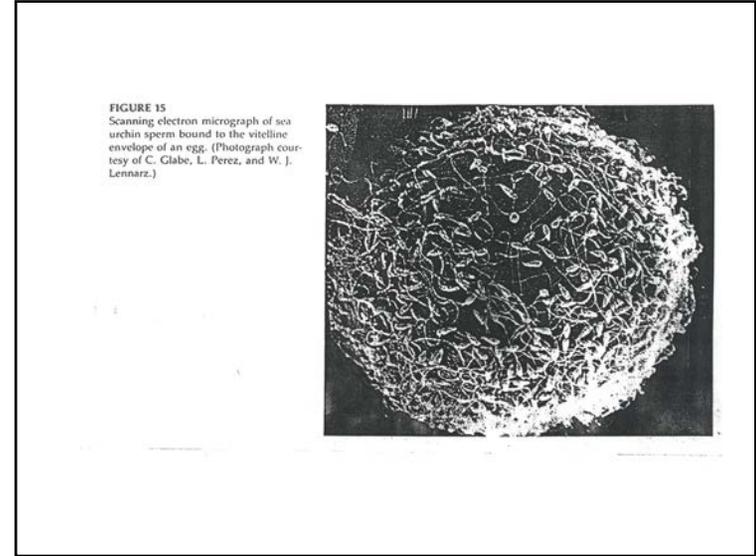
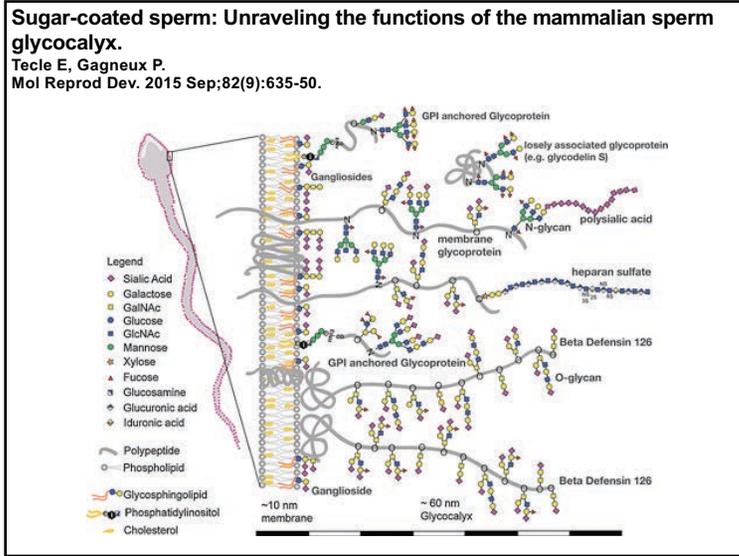
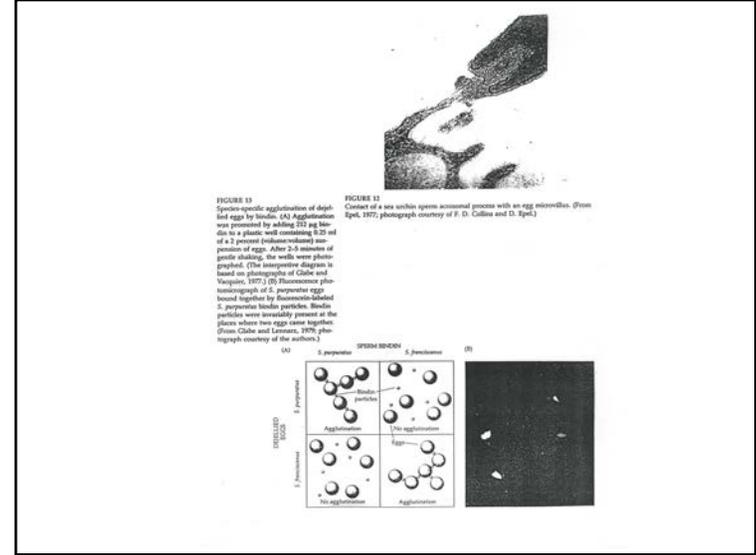
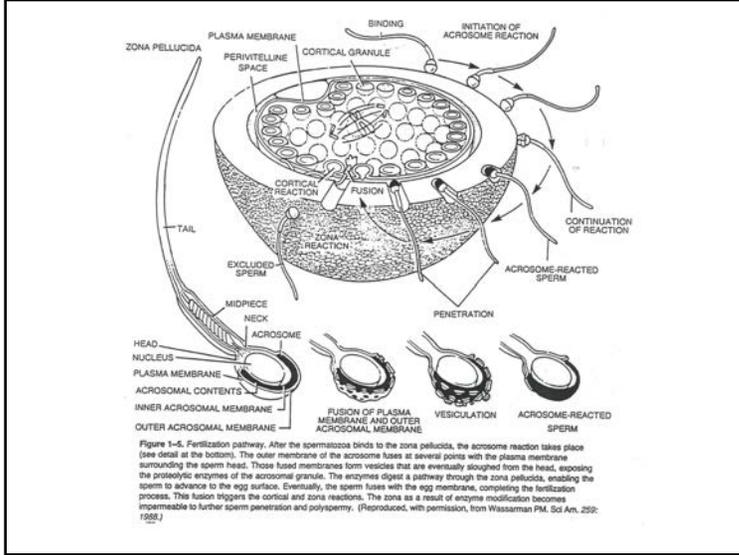
FIGURE 4. Trailing of the beating pedicels of hyperactivated mammalian spermatozoa in sea. In mouse, a, guinea hamster. The size normal between acrosome stages is 550 nm in sea and 1100 nm in sea. (a) Reference from Cameron, J. M., *Genes Dev.*, 3, 51, 1989. (b) Reference from Whit, D. E., Hargrove, E. S., and Shapiro, J. J., *Cell Biol.*, 103, 1073, 1986. (c) Reference from Shapiro, J. J., unpublished data, 1989.

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

Age (hours) of acrosome-reacted sperm at insemination	% Acrosome-reacted sperm in the population which are		% Eggs fertilized	
	Motile	Hyperactivated	Zona-intact	Zona-free
1	100	95	100	—
2	95	90	98	—
3	92	90	93	—
4	90	0	0	100
6	57	0	0	94
8	—	0	—	0
10	0	0	—	0

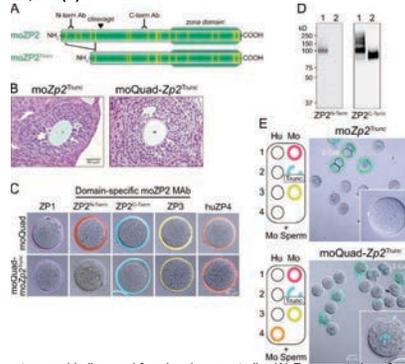
\*From Fleming and Yanagimachi (1958). Guinea-pig spermatozoa were induced to undergo a synchronous acrosome reaction. A population of 100% acrosome-reacted spermatozoa was isolated and incubated for up to 10 hr before they were mixed with zona-intact or zona-free guinea-pig eggs. Note that (a) there 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.



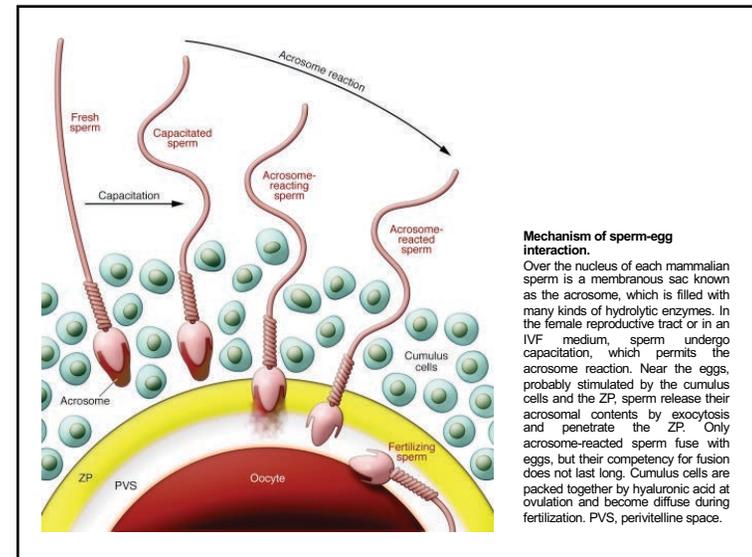
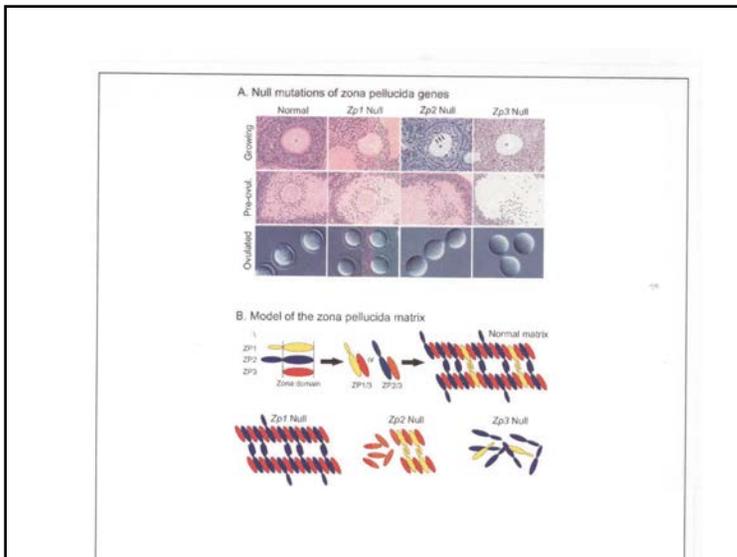
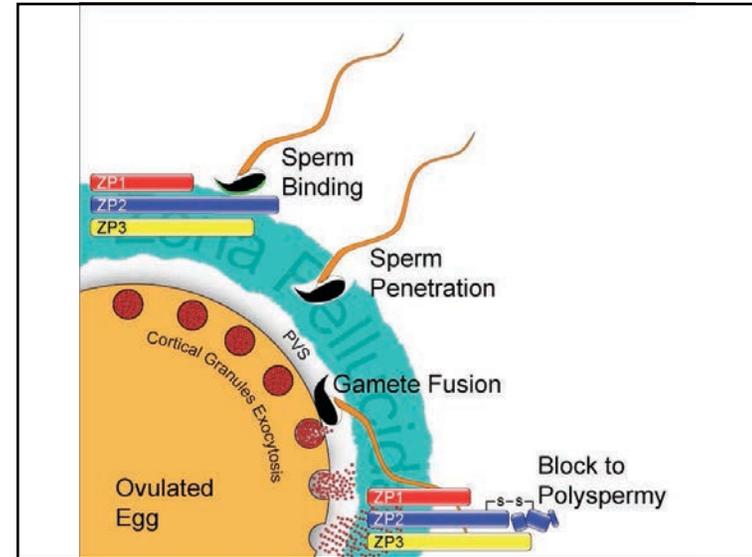


**A single domain of the ZP2 zona pellucida protein mediates gamete recognition in mice and humans.**

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.



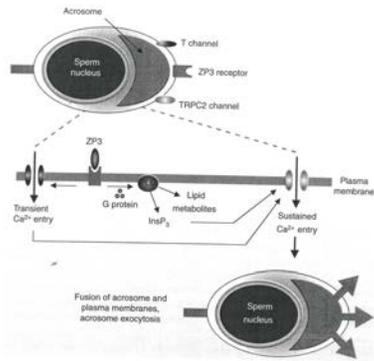


Figure 2 **Triggering of the acrosome reaction.** A model of the events involved in ZP3 signal transduction is shown. In the sperm head, TRPC2-ZP3 receptor activation during adhesion to the zona pellucida results in calcium entry through T-type channels, causing a transient elevation of cytosolic calcium concentration and activation of PLC through a G<sub>i</sub> and/or G<sub>q</sub> protein-mediated pathway, resulting in the production of InsP<sub>3</sub> 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.

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

Species	Name	Structure	Actions
Sea urchin	FSG*	Fucose sulfate glycoconjugate	↑Ca <sup>2+</sup> , ↑Na <sup>+</sup> ↑H <sup>+</sup> , K <sup>+</sup> release ↑pH <sub>i</sub> ↑Adenyl cyclase ↑cAMP ↑Protein kinase A ↑IP <sub>3</sub> ↑Phospholipase D ↑Phosphatidate
Starfish	ARIS*	Fucose sulfate glycoconjugate	↑Ca <sup>2+</sup> , ↑Na <sup>+</sup> ↑H <sup>+</sup> , K <sup>+</sup> release ↑pH <sub>i</sub> ↑cAMP (only in presence of CoARIS)
	CoARIS*	Sulfated steroidal saponins	Cofactor for ARIS
Mouse	ZP3*	Glycoprotein	G <sub>i</sub> activation ↑Ca <sup>2+</sup> ↑pH <sub>i</sub> ↑cAMP

\* SeGall and Lennarz, 1979, 1981; Garbers and Kopf, 1980; Garbers et al., 1983; Trimmer and Vacquier, 1986.

\* Ikadai and Hoshi, 1981a,b; Matsui et al., 1986a,b; Nishiyama et al., 1987a; Hoshi et al., 1990.

\* Wassarman, 1988, 1990; Kopf and Gerton, 1990; Florman and Babcock, 1990.

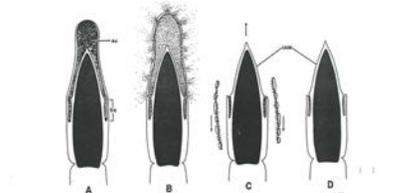


FIG. 9. Diagrams showing the progression of a typical acrosome reaction. (AG Acrosomal cap; EG equatorial segment of the acrosome; SAM inner acrosomal membrane. (From ref. 333, slightly modified).

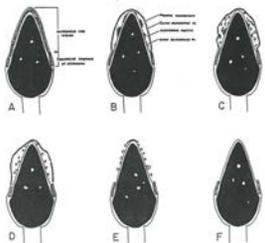
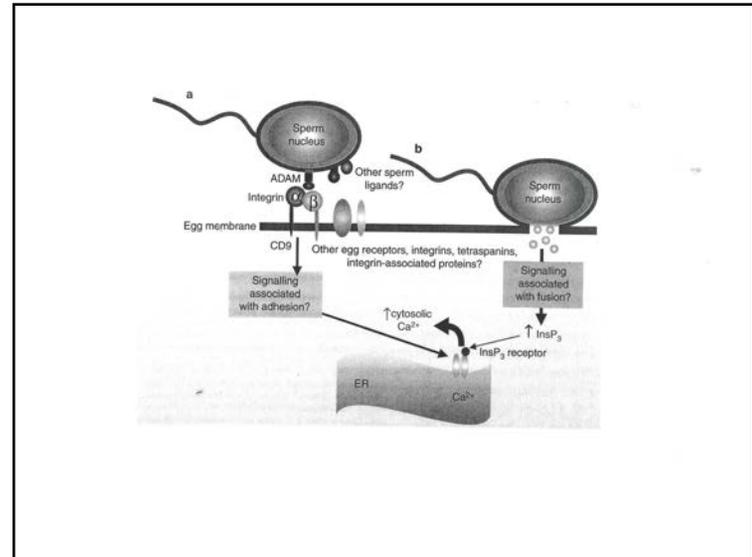
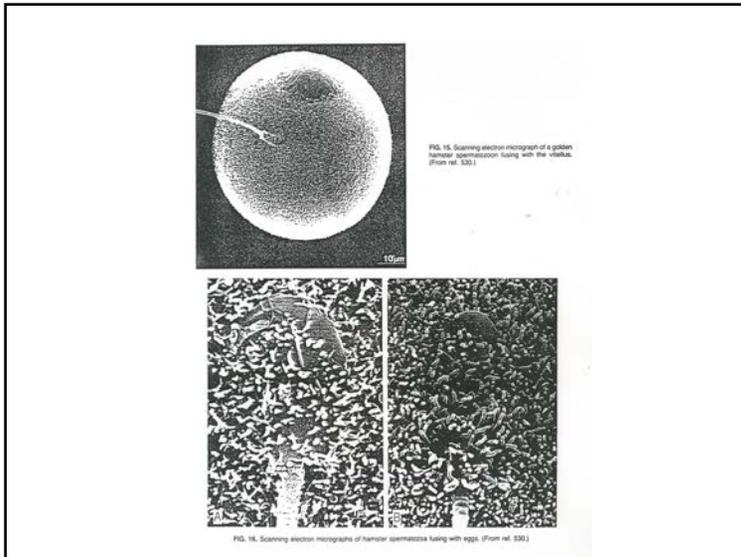
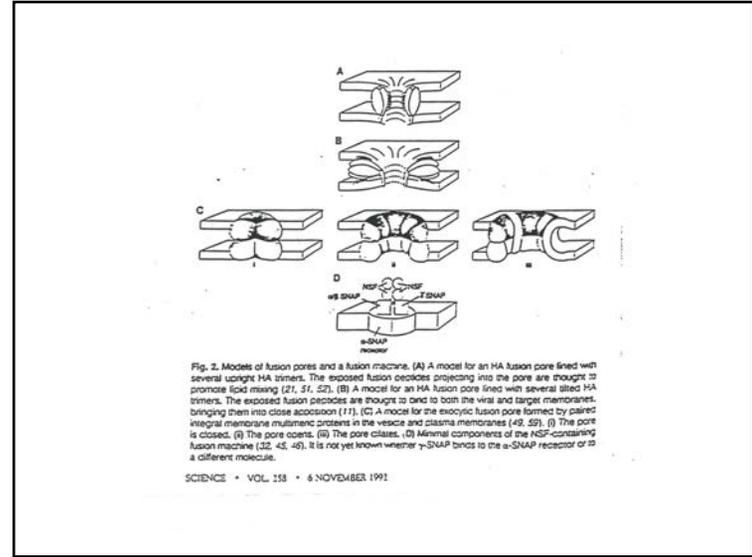
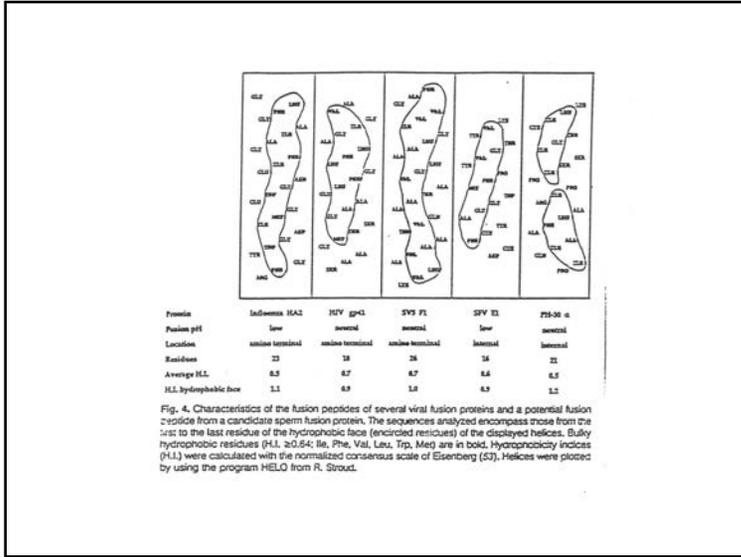


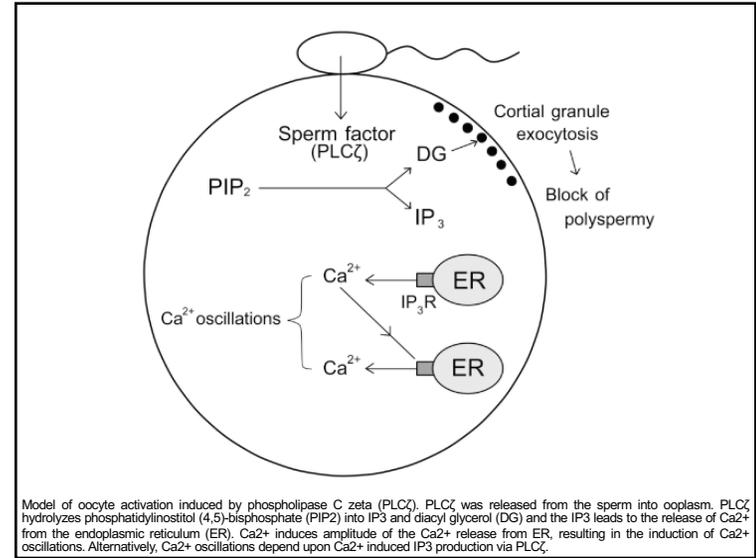
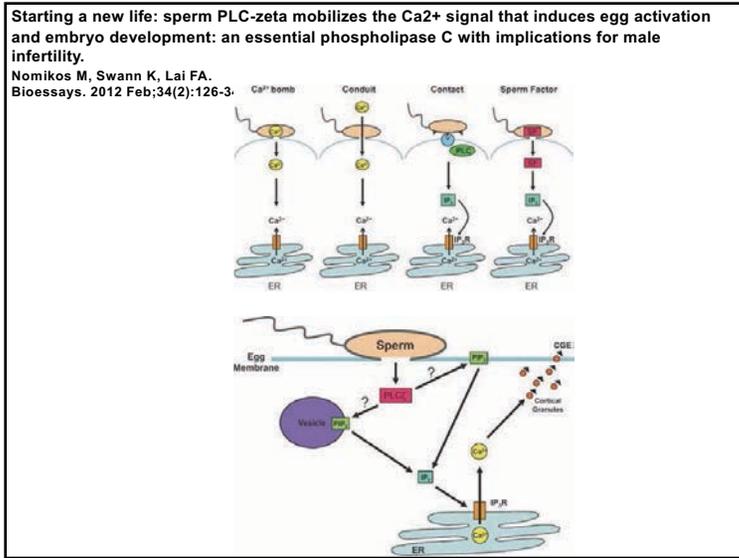
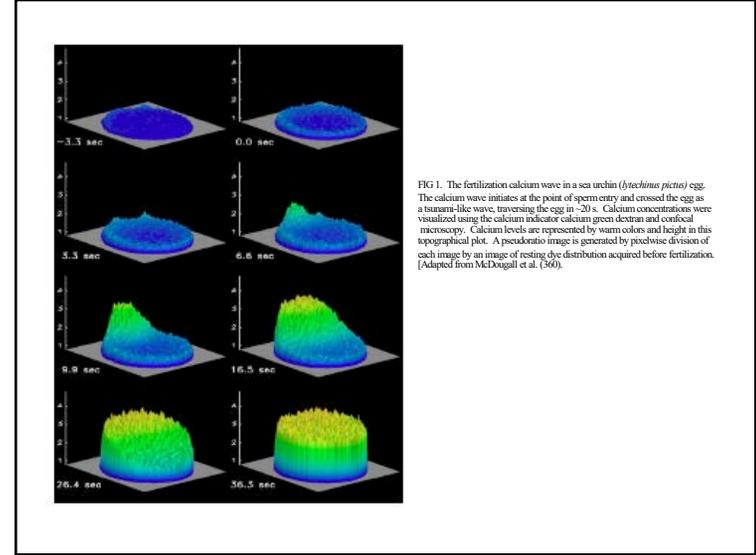
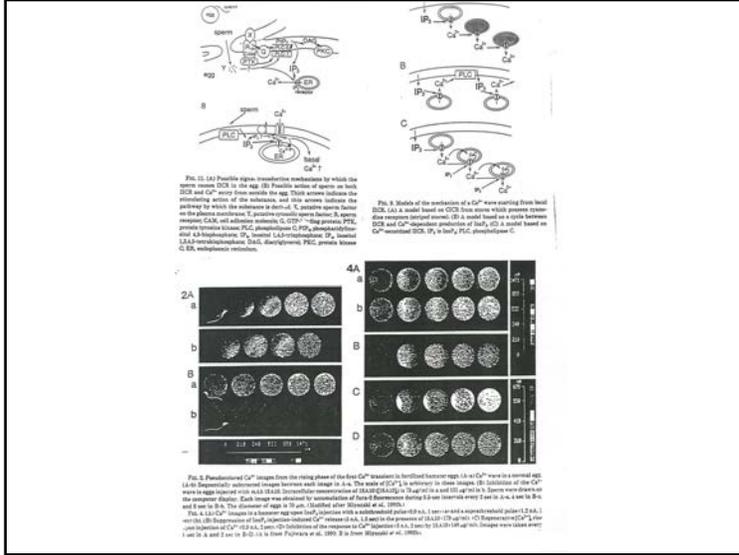
FIG. 10. A possible process of the acrosome reaction in human spermatozoa. (From ref. 348.)

TABLE 2. Enzymes reported to be of acrosomal origin

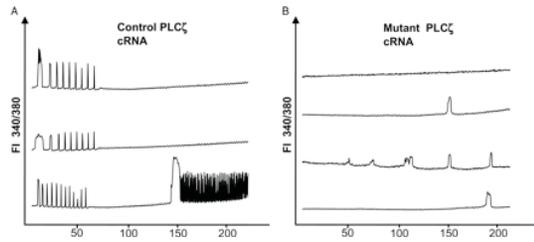
First reported before 1980	First reported after 1980 (references)
Hyaluronidase	β-N-Acetylhexosaminidase (454)
Acrosin	β-Galactosidase (454)
Proacrosin	β-Glucuronidase (454)
Acid proteinase	α-L-Fucosidase (454)
Esterase	Phospholipase C (453)
Neuraminidase	Cathepsin D (456)
Phosphatase	Peptidyl peptidase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



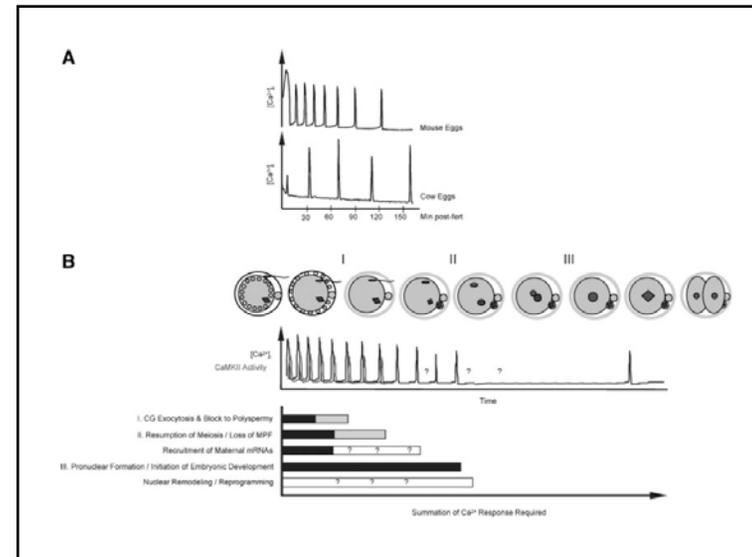
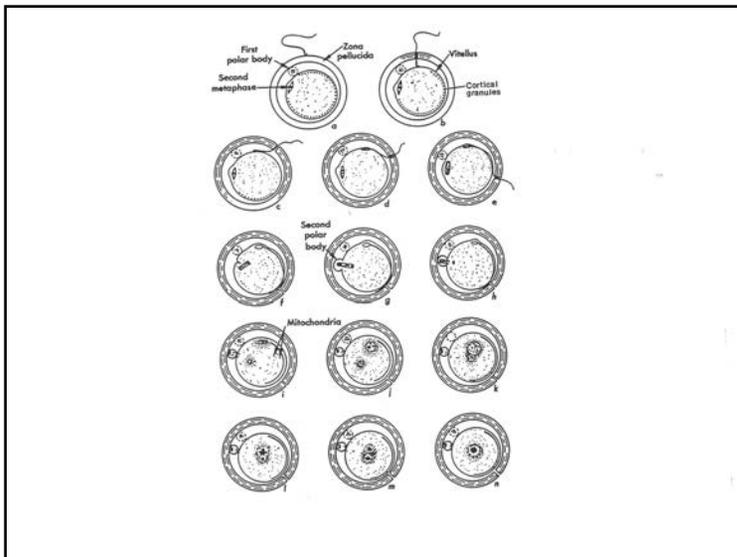
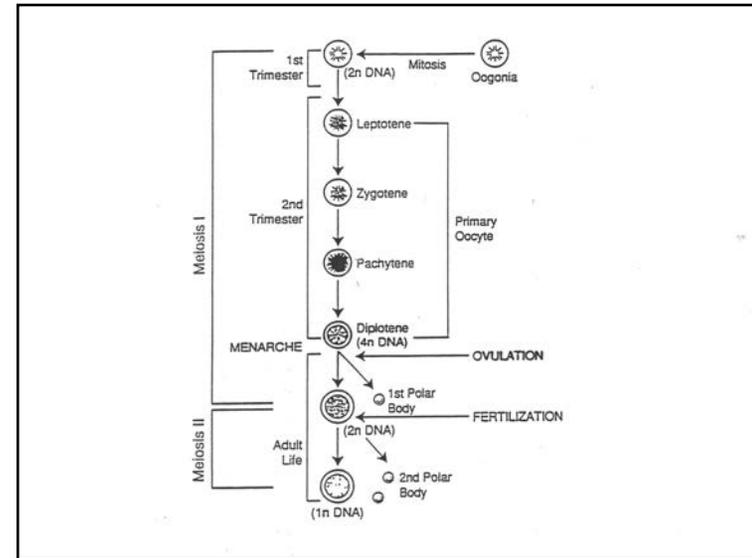


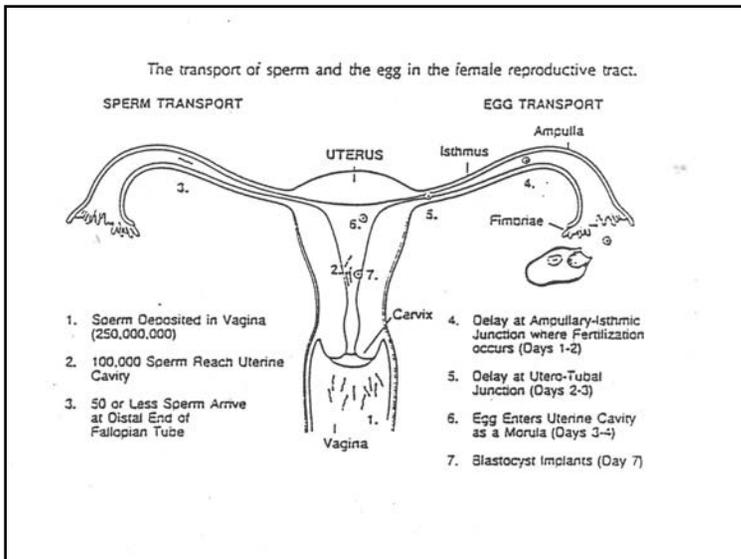
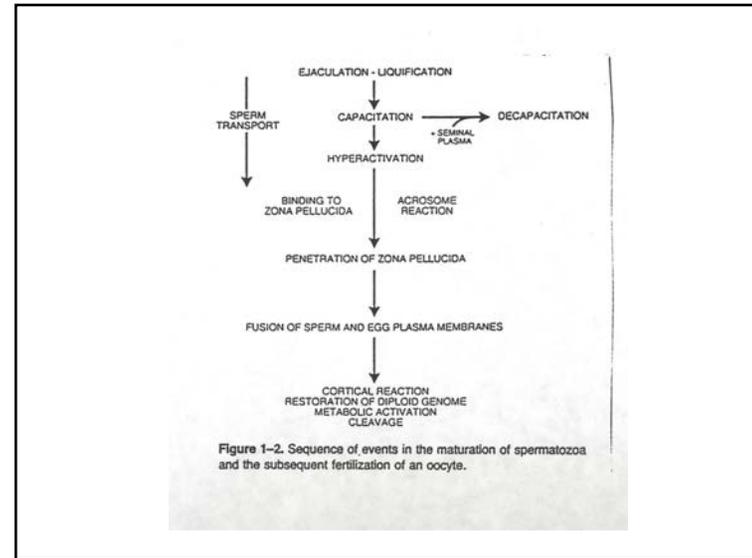
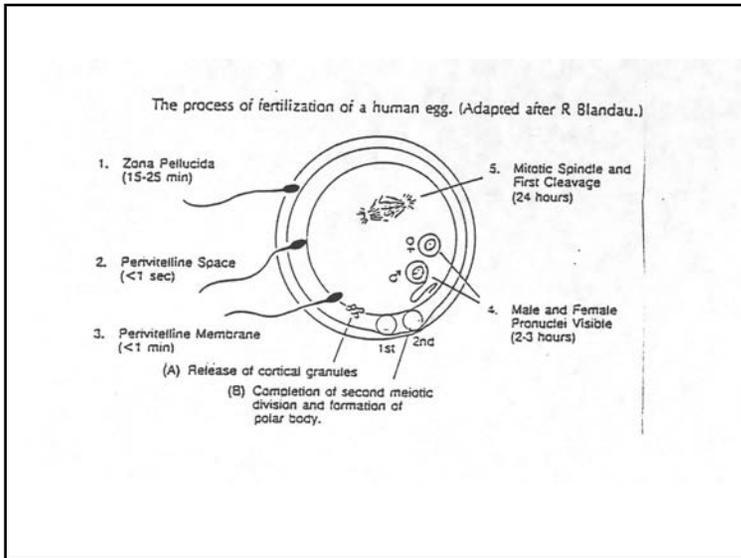


**Reduced amounts and abnormal forms of phospholipase C zeta (PLCzeta) in spermatozoa from infertile men.**  
 Heytens E, et al.  
 Hum Reprod. 2009 Oct;24(10):2417-28.

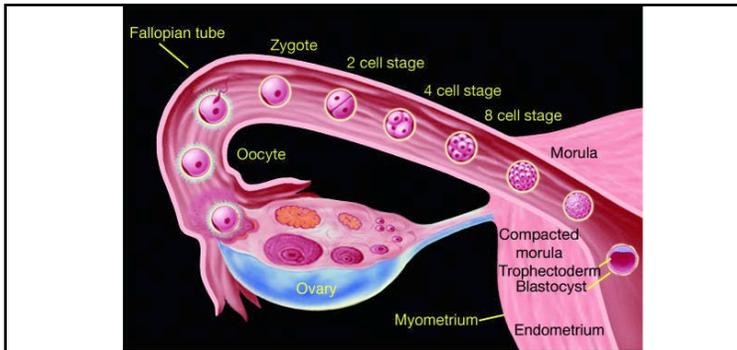


Microinjection of wild type control (A) and mutated (B) PLC  $\zeta$  into mouse oocytes and analysis of resultant intracellular calcium release.



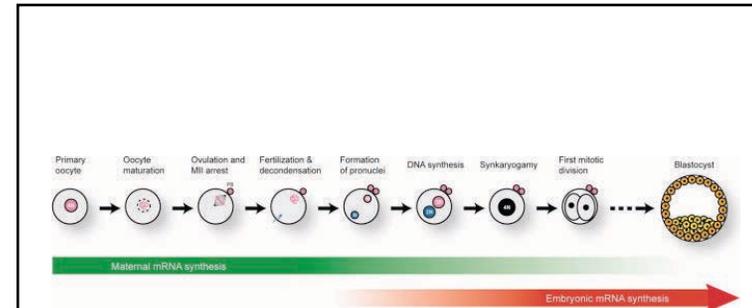


Preimplantation embryo

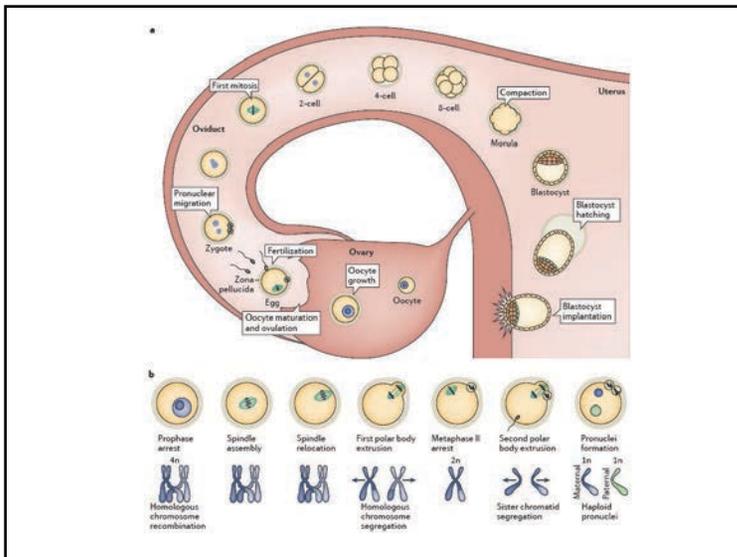


**Figure 1.**

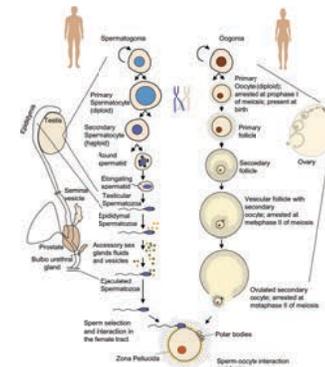
The early stages of human development from fertilization to blastocyst formation. Fertilization occurs in the fallopian tube within 24 to 48 hours of ovulation. The initial stages of development, from fertilized ovum (zygote) to a solid mass of cells (morula), occur as the embryo passes through the fallopian 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 trophoblast. 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 syngamy 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 trophoblast (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.



**The contribution of human sperm proteins to the development and epigenome of the preimplantation embryo.**  
Castillo J, Jodar M, Oliva R.  
Hum Reprod Update. 2018 Sep 1;24(5):535-555

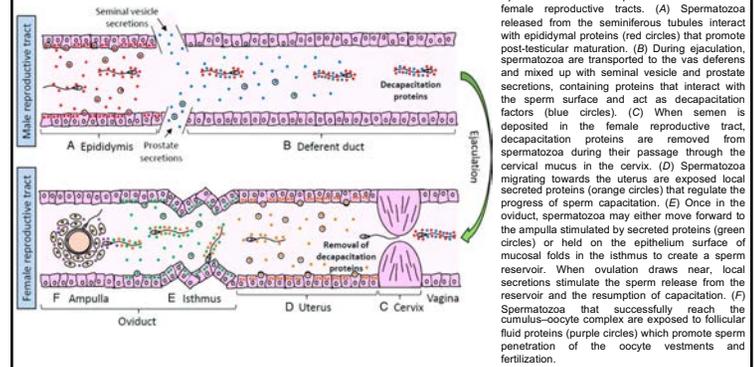


**Ram seminal plasma and its functional proteomic assessment.**  
Leahy T, Rickard JP, Bernecic NC, Druart X, de Graaf SP.  
Reproduction. 2019 Jun;157(6):R243-R256.

**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	CESSA	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-vbeta-3 integrin receptor. Calcium binding

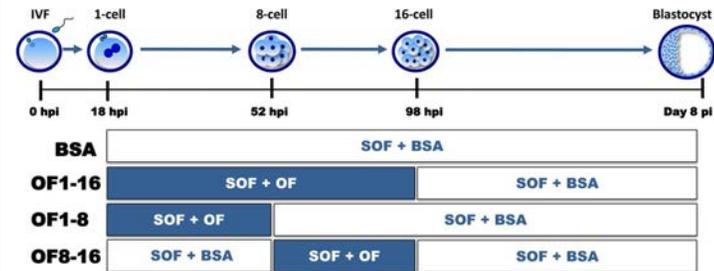
**Proteins from male and female reproductive tracts involved in sperm function regulation.**  
Hernández-Silva G, Chirinos M.  
Zygote. 2019 Feb;27(1):5-16.

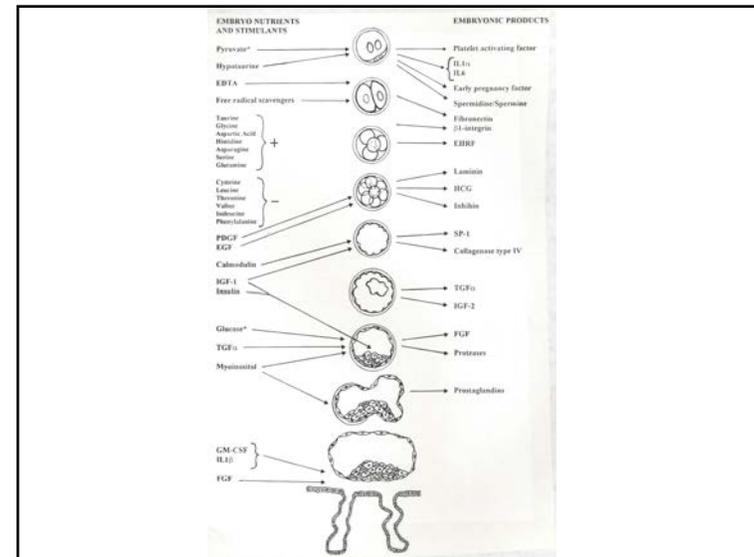
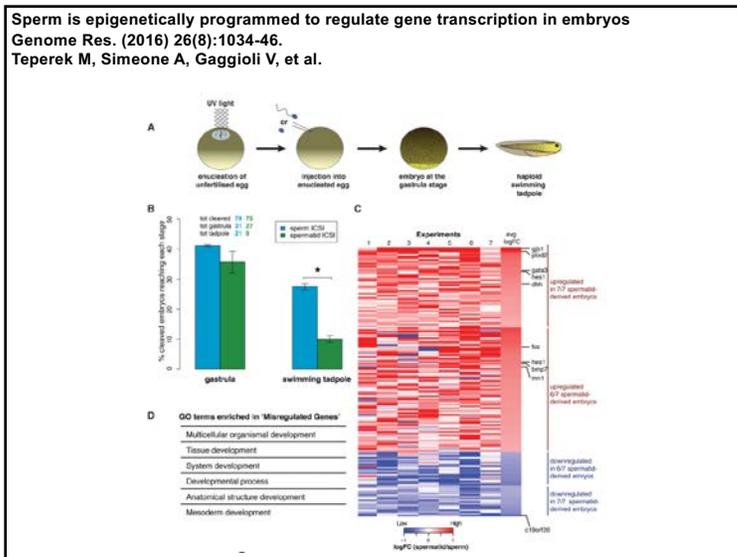
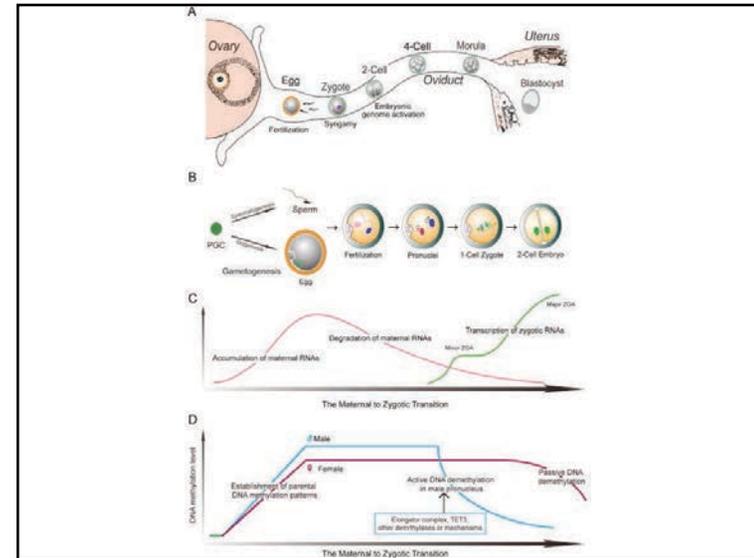
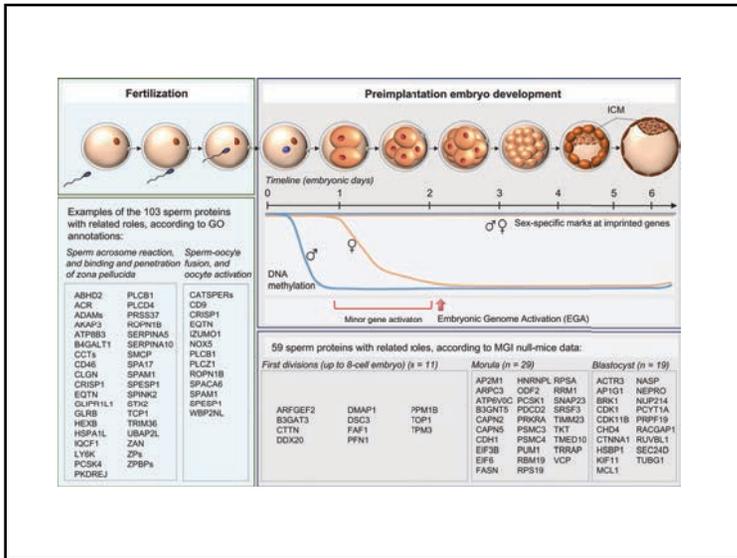


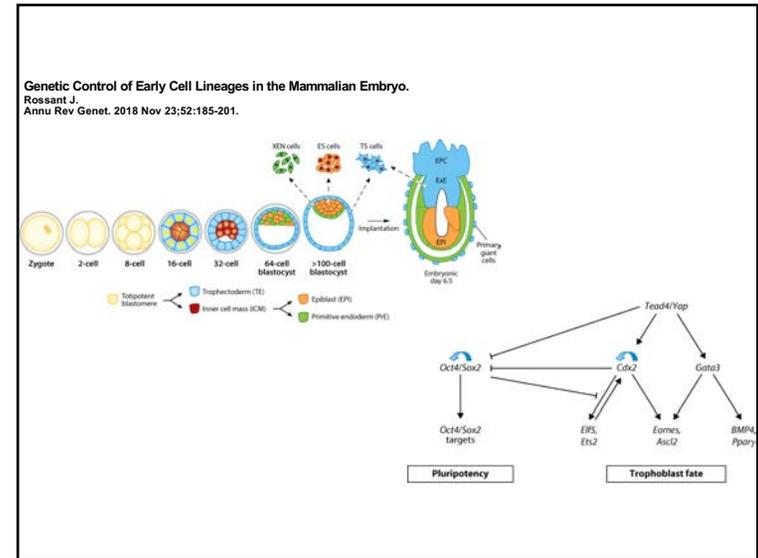
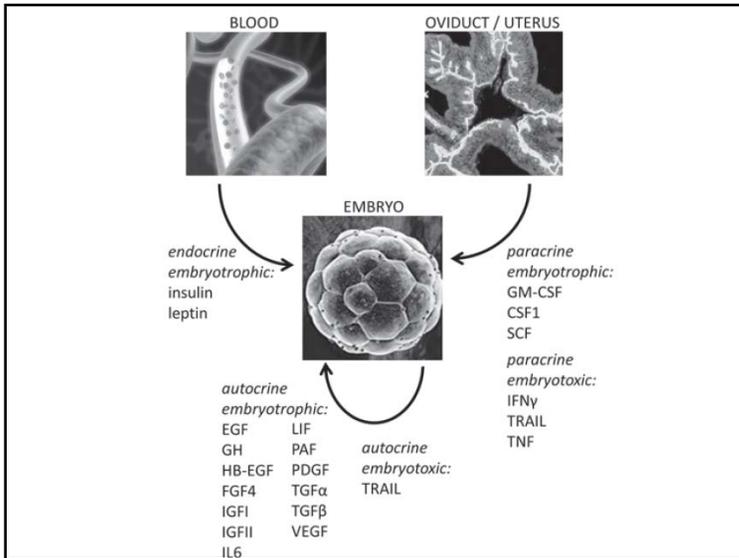
**Table II** Subgroups and examples of sperm proteins that could modulate gene expression. A total of 560 sperm proteins have been identified as potential modulators of gene expression according to their association with specific GO annotations (see Supplementary Table 4). The 560 proteins have been sub-classified into four subgroups according to their function: (1) Transcription factors or other gene expression related proteins (381 proteins); (2) Proteins that may regulate the genome methylation and demethylation patterns (25 proteins); (3) Proteins able to modulate several histone post-translational modifications (PTMs; 118 proteins) and (4) Proteins involved in the biogenesis, processing and functions of the small non-coding RNAs (snRNAs; 36 proteins).

Subgroups of sperm proteins related to the regulation of gene expression (n = 560) and the corresponding role (GO annotations)	Examples of specific sperm delivered proteins
1. Transcription factors or gene expression-related proteins (n = 381)	
Transcription factors	ZHX2, GTF3C3, ATF5, ZNF219, MYF2, SLM, CSRN2, GZF1, STAT1, STAT6
Regulation transcription factor import	IPO11
Termination of transcription	DHX38, CPSF2, CPSF3, CPSF2
2. DNA methylation regulators (n = 25)	
DNA demethylation	APEX1, CtBP1, APOBEC3C
DNA methylation	TRIM28, USP7, MPHOSPH8, DNMT1
Imprinting regions	GNAS
3. Histone PTMs regulators (n = 118)	
Acetylation	BLP1, WDR5, CBK1
Deacetylation	HTA1, HDX1, HDAC1
Phosphorylation	PRKCD, PRKAA1
Diphosphorylation	PRH1F, PPP5C
Methylation	MEN1, NASP, SUZ12
Demethylation	KDM9B, JHD1C
Ubiquitination	RNF40, CUX4B
4. ncRNA biogenesis, processing and function regulators (n = 36)	
ncRNA biogenesis	SNP1, BMRP1A, GTF2B, GTF3C1, NATC4, CPSF3
ncRNA processing	PRKRA, HNRNPA2B1, SIRT, DROSHA
ncRNA function	CHOT1, EIF6, TNRC1A, AGO3

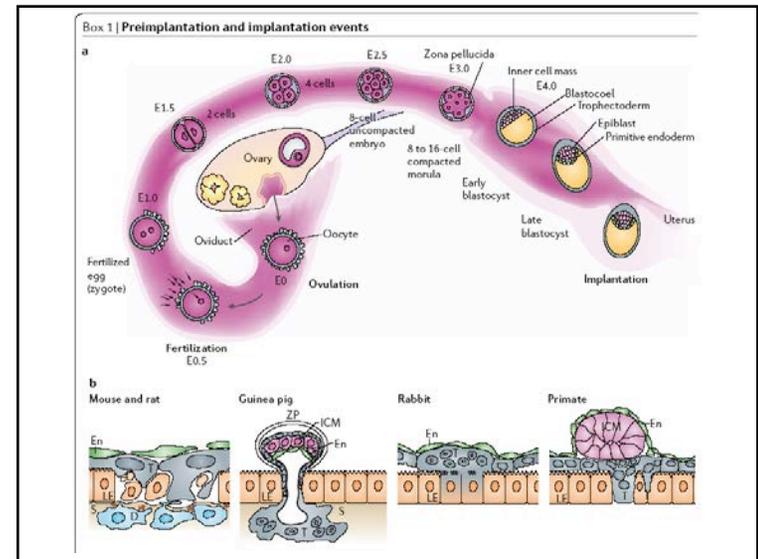
**Embryo culture in presence of oviductal fluid induces DNA methylation changes in bovine blastocysts.**  
Reproduction. 2017 Jul;154(1):1-12.  
Barrera AD, García EV, Hamdi M, Sánchez-Calabuig MJ, López-Cardona AP, Balvis NF, Rizo D, Gutiérrez-Adán A.

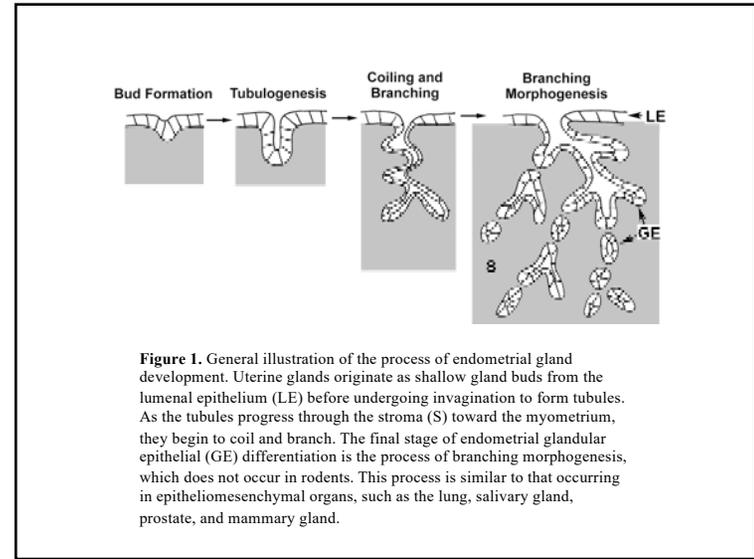
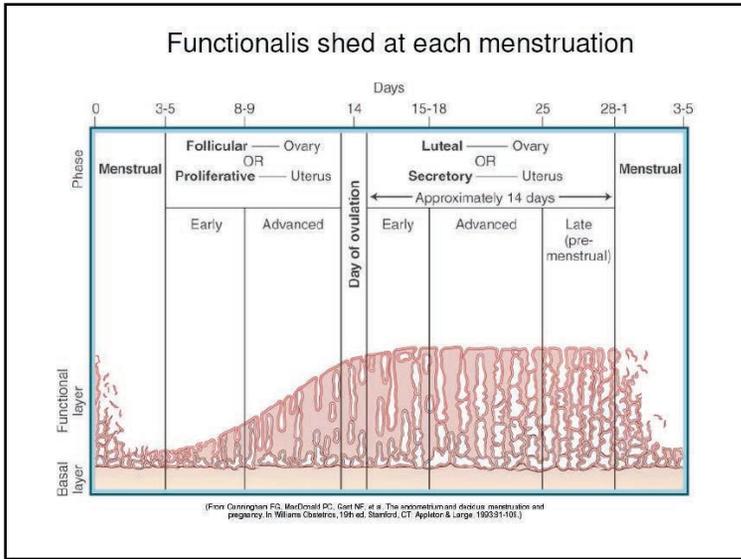
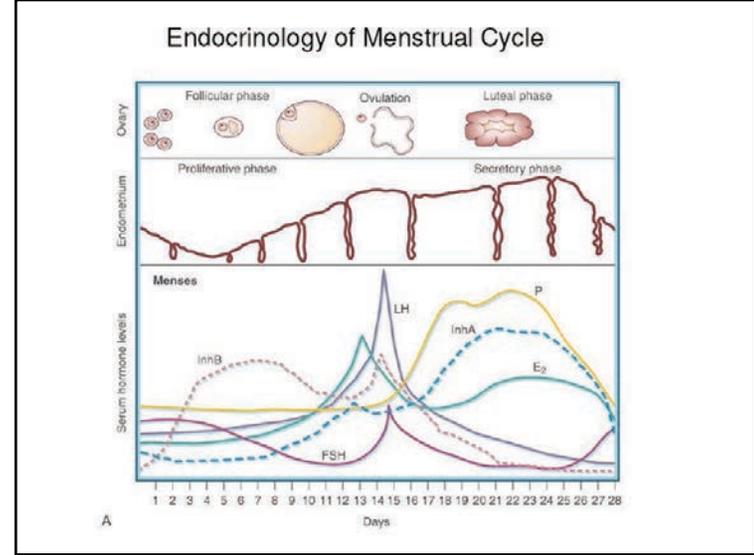
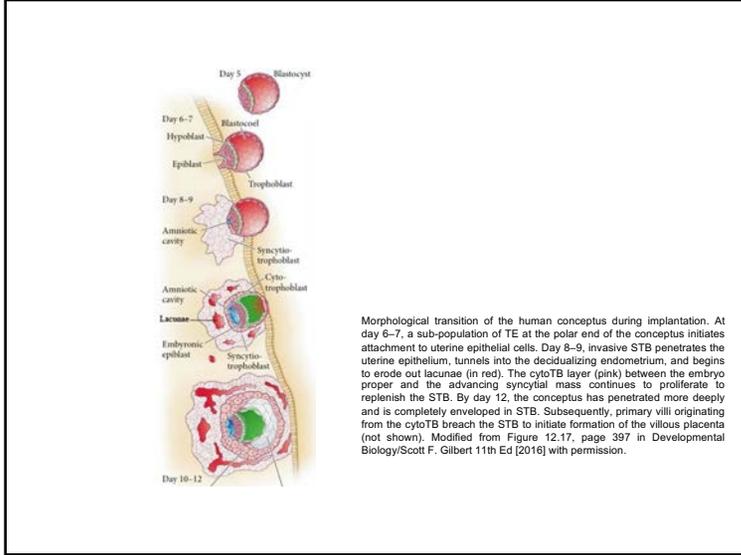


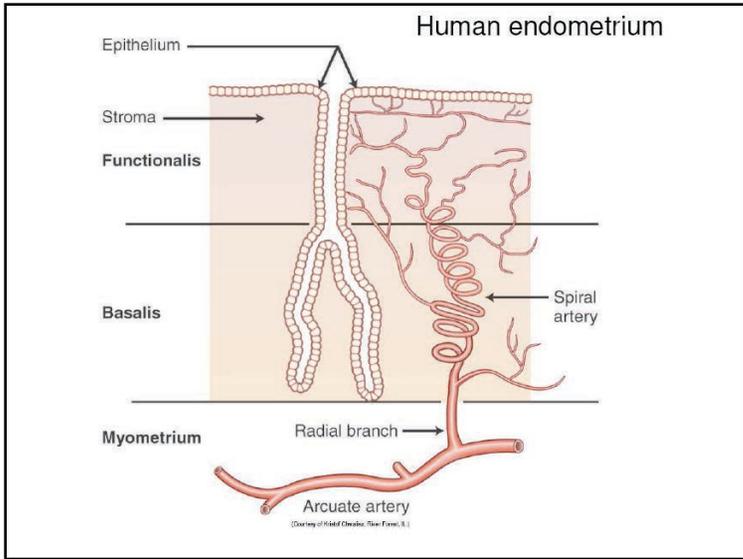
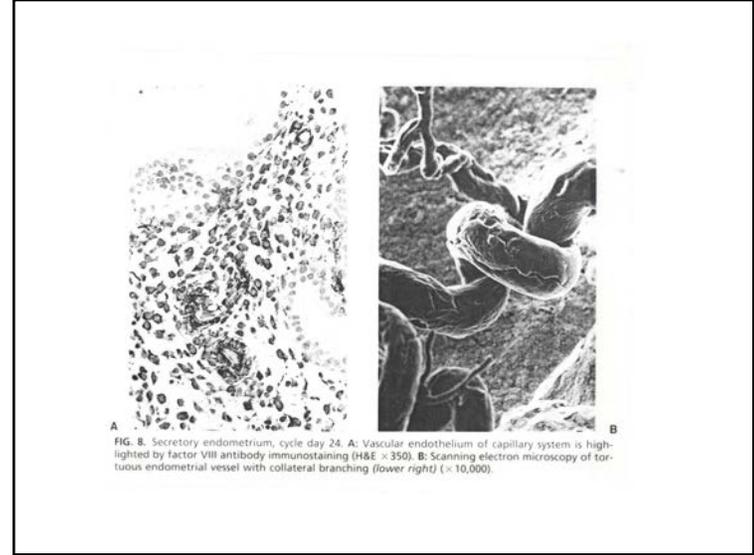
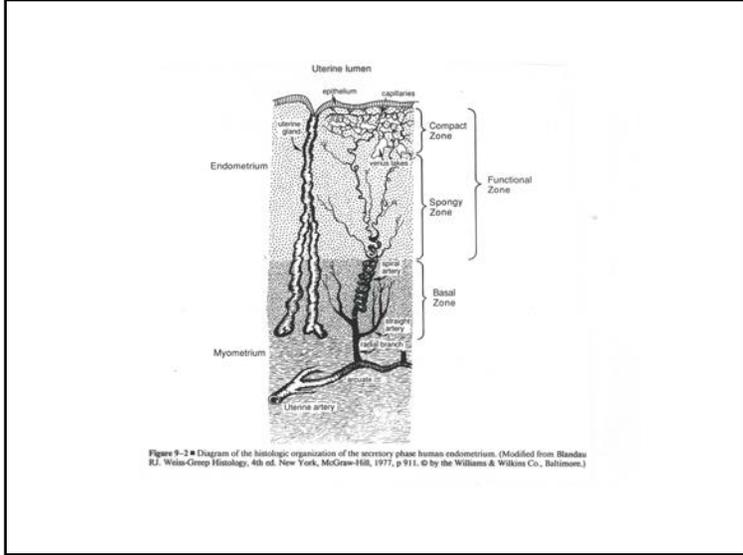




# Implantation







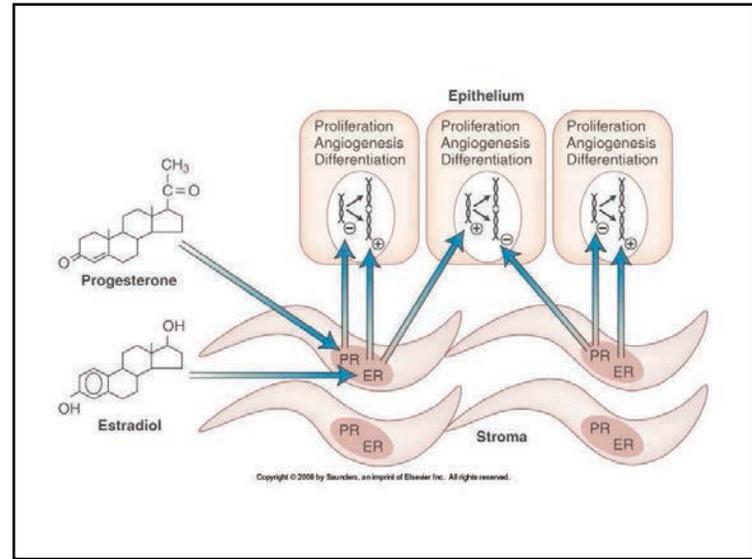
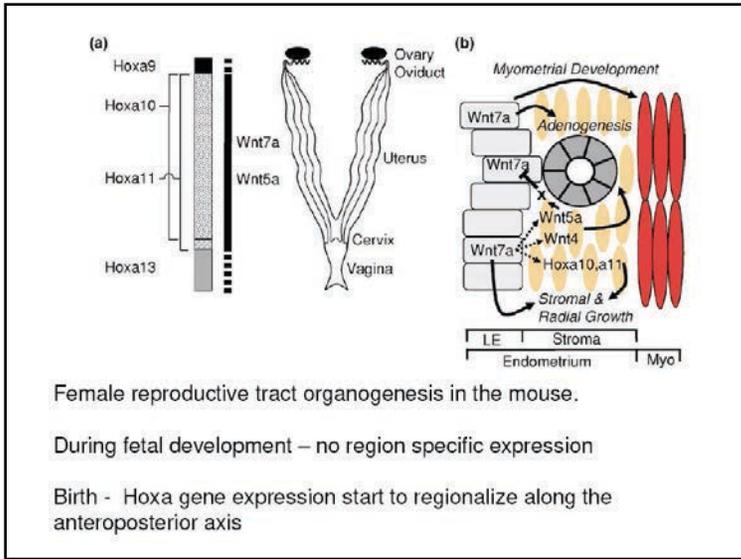
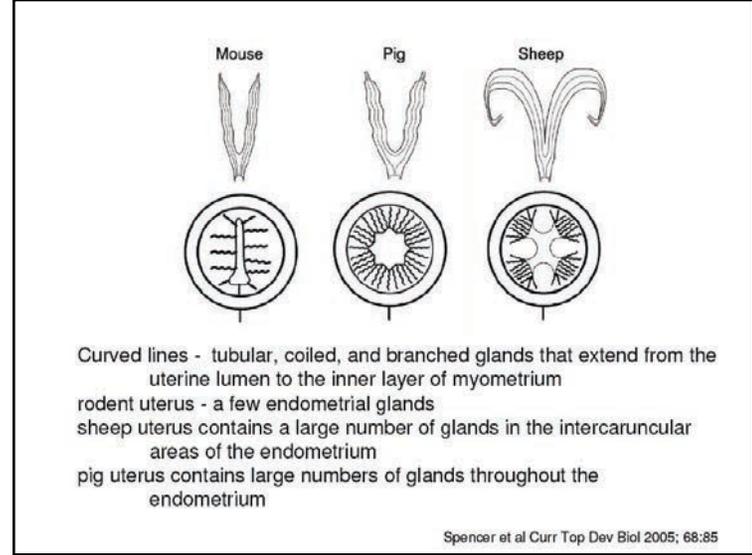
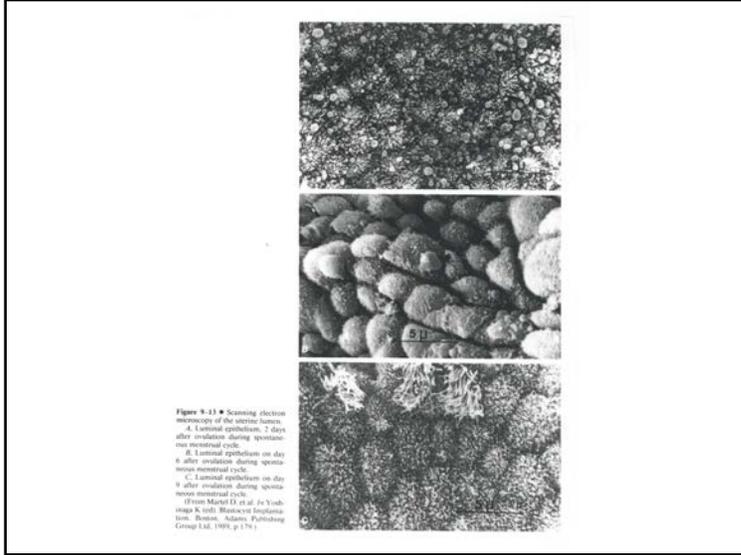
## Female Reproductive Anatomy

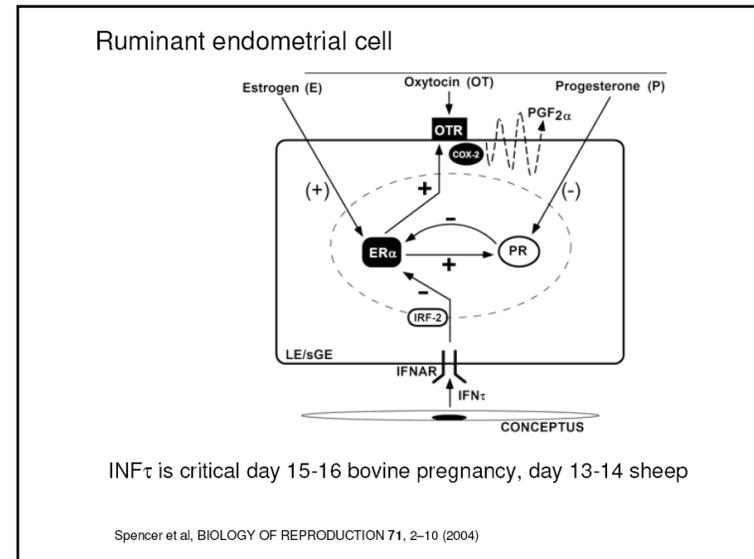
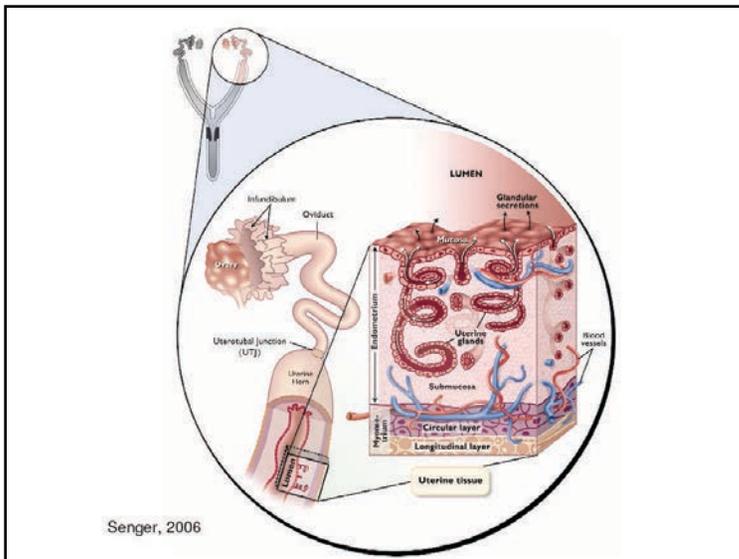
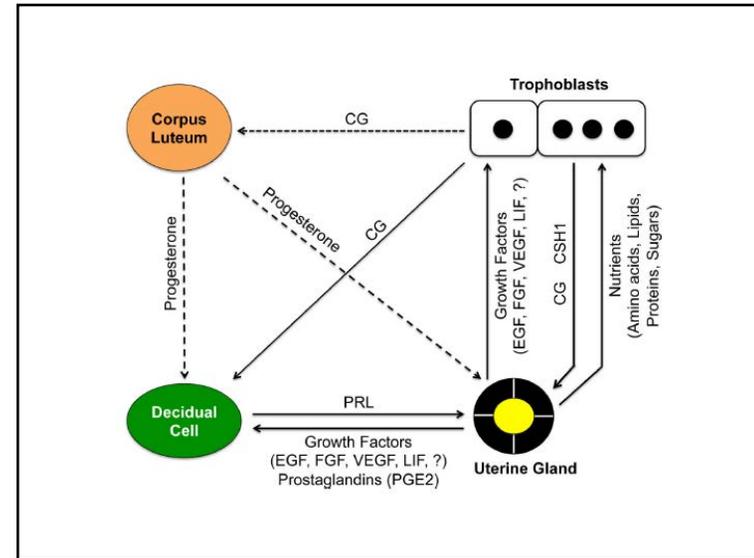
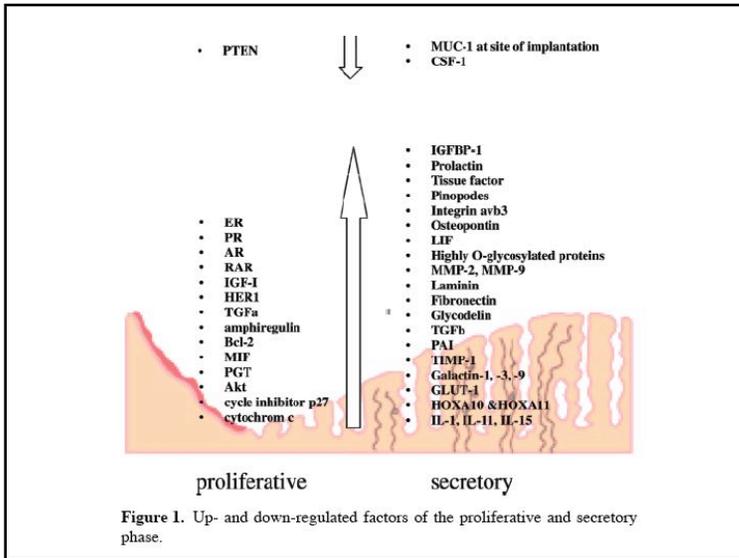
### Uterus

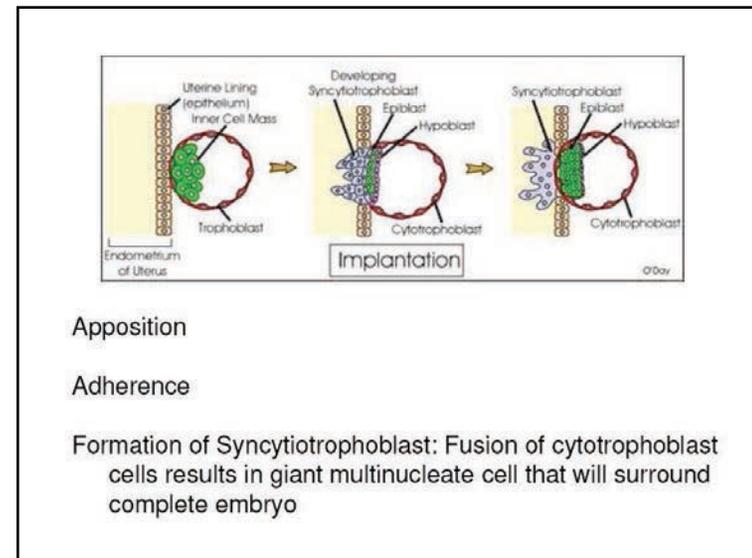
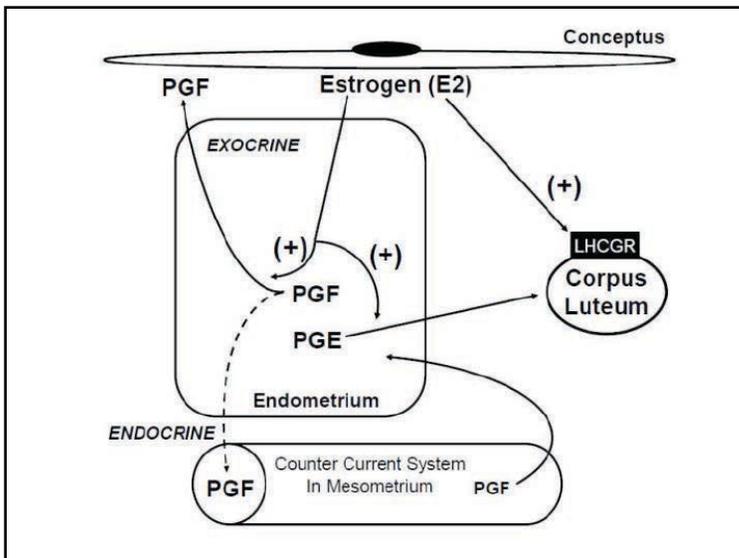
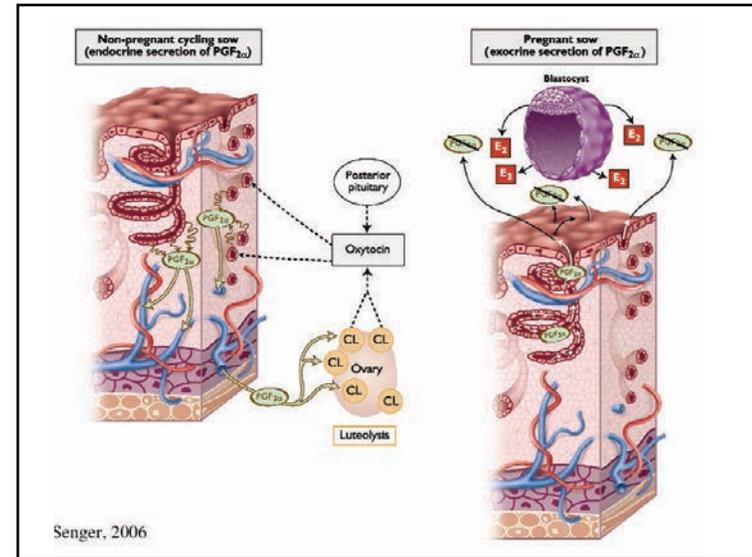
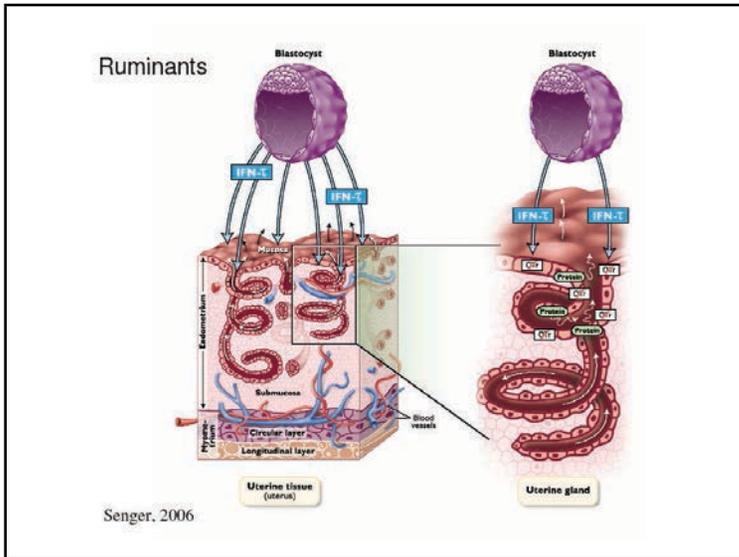
Prominent organ of the female reproductive tract, organ of pregnancy

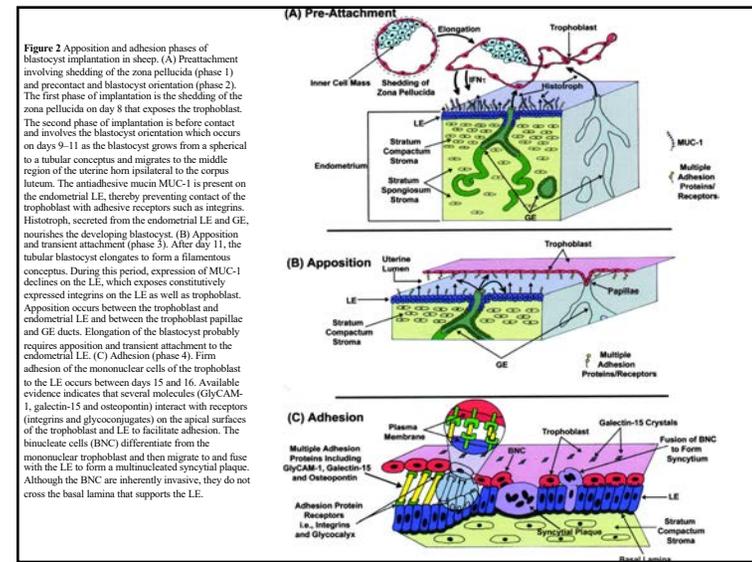
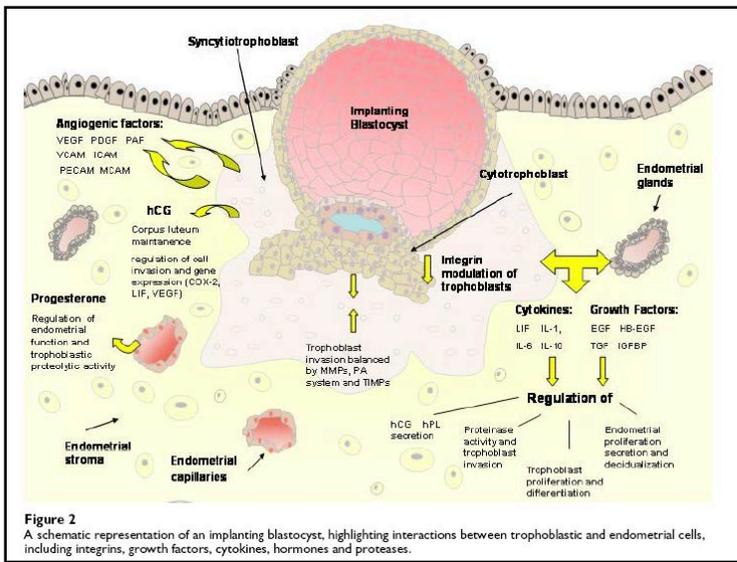
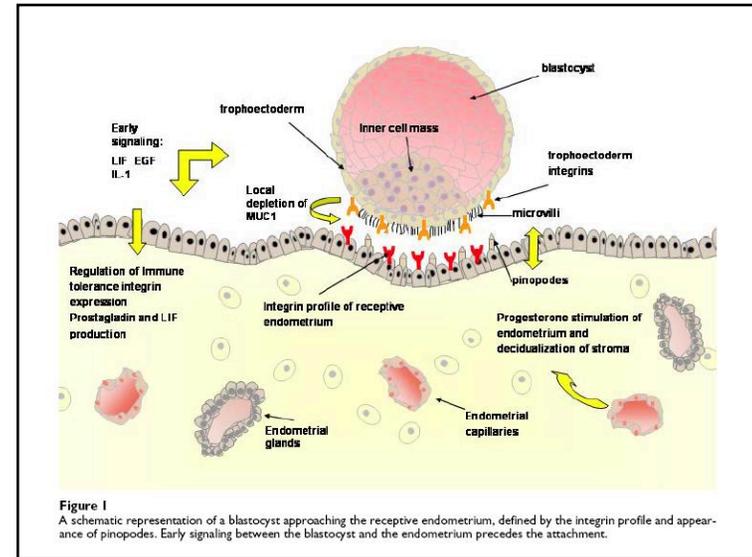
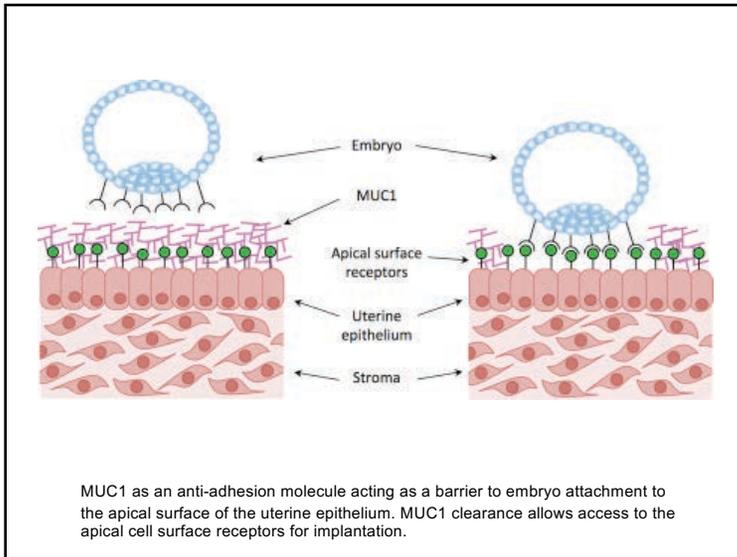
1. serosa – perimetrium
2. muscularis – myometrium
3. mucosa – endometrium

The image shows a cross-section of the uterus. The outer layer is the Perimetrium, the middle layer is the Myometrium, and the inner layer is the Endometrium. The Lumen is the central cavity of the uterus.



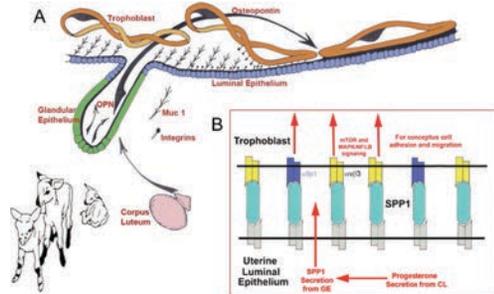






**Osteopontin: a leading candidate adhesion molecule for implantation in pigs and sheep.**

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  $\alpha v \beta 3$  integrin receptor on trophoblast as a binding partner for OPN[66]. OPN then likely acts as a bridging ligand between  $\alpha v \beta 3$  on trophoblast and as yet unidentified integrin receptor(s) expressed on the opposing uterine LE. Note that the  $\alpha 5$  integrin subunit was immunoprecipitated from membrane extracts of biotinylated  $\alpha T r 1$  cells that were eluted from an OPN-Sepharose column, but the  $\beta 1$  integrin subunit, the only known binding partner for  $\alpha 5$ , could not be immunoprecipitated. Therefore, while we cannot definitively state that OPN binds  $\alpha 5 \beta 1$  integrin on  $\alpha T r 1$ , we are reticent to exclude this possibility.

**Cytokines critical for implantation**

LIF – uterine gland expression and by stromal cells that surround the blastocyst during attachment

1. Uterine preparation
2. Attachment

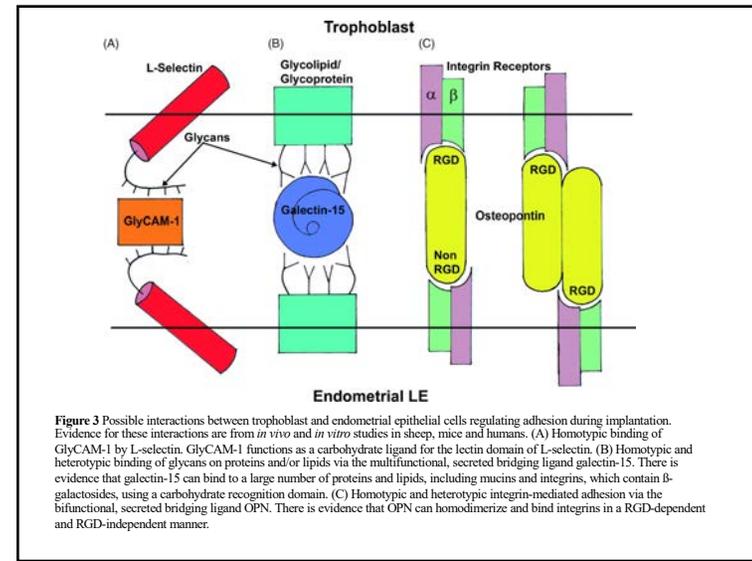
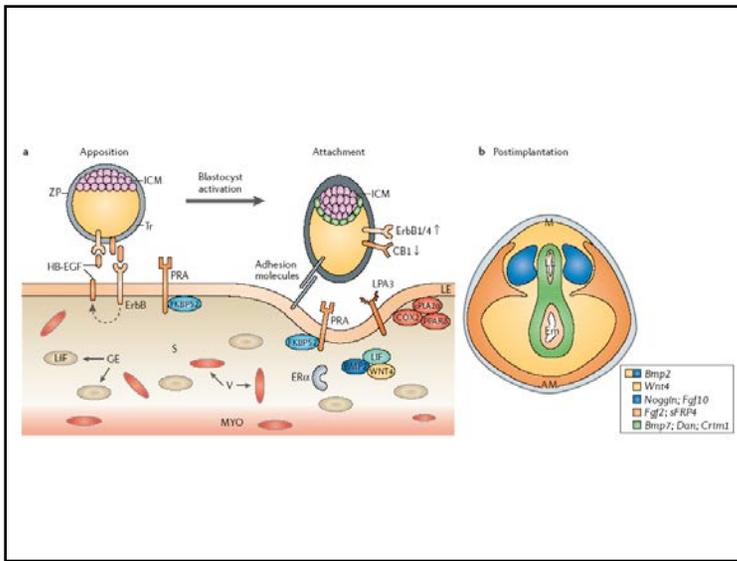
Lif  $-/-$  mice: blastocysts remain dormant, do not implant

Molecular mechanisms unclear

Gp130 – mutate the STAT binding site results in implantation failure

LIF – high expression in human uterine tissue

Homeobox proteins – Hoxa10, Hoxa11

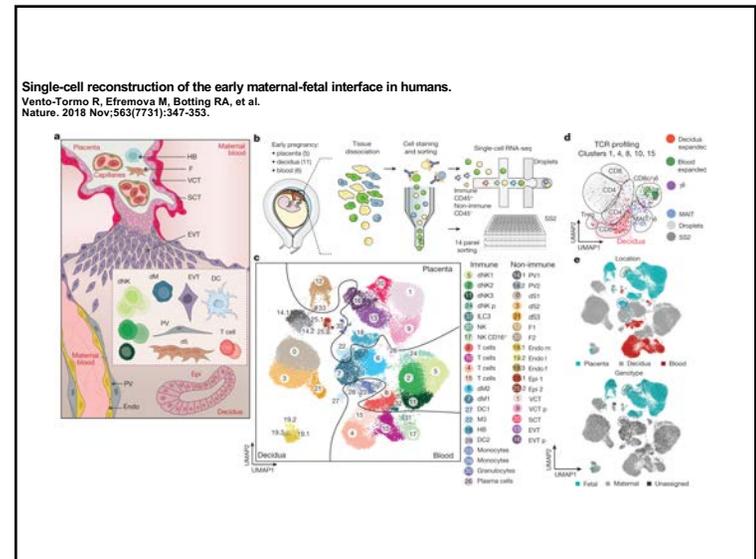
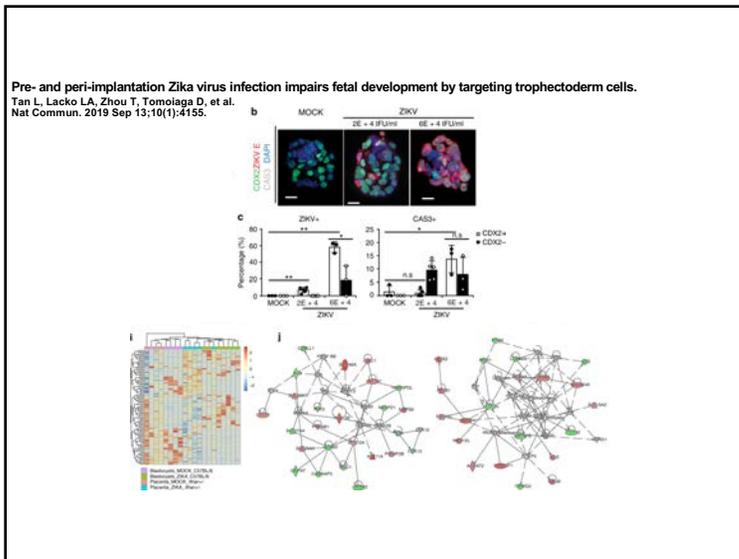
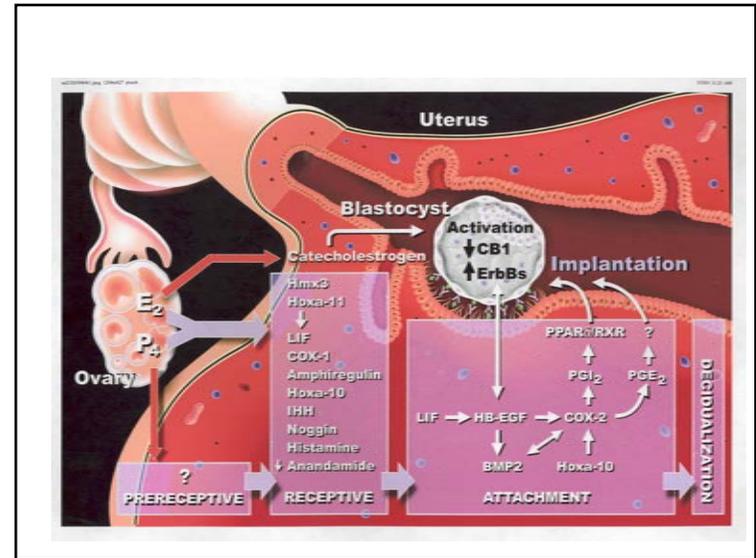


**Figure 3** Possible interactions between trophoblast and endometrial epithelial cells regulating adhesion during implantation. Evidence for these interactions are from *in vivo* and *in vitro* studies in sheep, mice and humans. (A) Homotypic binding of GlyCAM-1 by L-selectin. GlyCAM-1 functions as 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  $\beta$ -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 <sup>1</sup>	Null/Conditional Phenotype	Reference
<i>Cdh1</i>	E-cadherin	LE and GE (GD1-4), stroma (GD4-5)	Embryonic lethal, implantation defect (conditional)	101,102
<i>Clec3</i>	chondro-chemo calcium activated 3	LE and GE (peaks on GD1)	Viable and fertile	103,104
<i>Cxcl13</i>	chemokine (C-X-C motif) ligand 13	GE, LE	Viable and fertile	105,106
<i>Fcyl1</i>	ferritin binding protein 1	GE (GD4-5)	N/A	44
<i>Foxo2</i>	forkhead box A2	GE (prenatal and adult)	Embryonic lethal, implantation defect (conditional)	35,307
<i>Gulo</i>	glucuronotransferase (L-) oxidase	GE→LE (GD4-5)	Viable and fertile	44,308
<i>Itih</i>	Itih-like Helicobacter	LE and GE (peaks on GD3-4)	Embryonic lethal, implantation defect (conditional)	109
<i>Irf8</i>	interleukin 6 signal transducer	GE (GD3-5) Decidua (GD7)	Viable and fertile	110,111
<i>Klf5</i>	Kruppel like factor 5	LE and GE (GD1-5) Decidua (GD4-5)	Embryonic lethal, implantation defect (conditional)	112
<i>Lap4</i>	leucine rich repeat containing G-protein-coupled receptor 4	GE, LE	Implantation defect	41
<i>Lif</i>	leukemia inhibitory factor	GE→Stroma (GD4)	Implantation defect	34
<i>Lif</i>	lacritransferrin	LE and GE (GD1-2)	Viable and fertile	113,114
<i>Lys2</i>	lysinease 2	Stroma, GE (GD1-5)	Viable and fertile (knock-in)	44,115
<i>Mxl1</i>	homeobox, msh-like 1	LE and GE (peaks on GD4 and declines)	Embryonic lethal, subfertility (conditional)	116,117
<i>Mxl2</i>	homeobox, msh-like 2	LE and GE (peaks on GD4)	Viable and fertile, infertile (double conditional)	116,117
<i>Prr138</i>	protease, serine, 38	GE (GD5-8)	N/A	118
<i>Prr139</i>	protease, serine, 39	GE (GD5-9)	N/A	119
<i>Pyp1</i>	Prostaglandin-endoperoxidase synthase 1	LE and GE (peaks on GD4)	Viable, delayed parturition	120,121
<i>Sltk2</i>	SRE3 domain and serine/threonine repeats 2	GE (GD4-5)	Viable and fertile	44,122
<i>Sltk2a2</i>	solute carrier family 21 (anion/cation transporters, member 2)	GE	Postnatal lethal	44,123
<i>Spm1</i>	serine protease inhibitor, Kazal type 3	GE (onset GD4)	Postnatal lethal	54,124
<i>Sulf1d1</i>	sulfotransferase family 1D, member 1	GE→LE (GD1-4)	N/A	44
<i>Tro</i>	Trophobin	LE and GE (peaks between GD4 and 6)	Viable and fertile	125
<i>Tw</i>	transferrin	GE only (peaks on GD4)	Viable and fertile	126,127

<sup>1</sup>GE, gestational day; GE, glandular epithelium; LE, luminal epithelium



**Schedule/Lecture Outline –**

January	14 & 16	Week 1	Systems Biology Introduction
	21 & 23	Week 2	Molecular/ Cellular/ Reproduction Systems
	28 & 30	Week 3	Sex Determination Systems
February	4 & 6	Week 4	Male Reproductive Tract Development & Function
	11 & 13	Week 5	Female Reproductive Tract Development & Function
	18 & 20	Week 6	Gonadal Developmental Systems Biology
	25 & 27	Week 7	Testis Systems Biology
March	3 & 5	Week 8	Ovary Systems Biology
	10 & 12	Week 9	Epigenetics and Transgenerational Gonadal Disease
	<b>16 – 20</b>	<b>Week 10</b>	<b>Spring Break</b>
	24 & 26	Week 11	Gametogenesis/ Stem Cells/ Cloning
	31 & 2	Week 12	Hypothalamus-Pituitary Development & Function
April	7 & 9	Week 13	Reproductive Endocrinology Systems
	14 & 16	Week 14	Fertilization & Implantation Systems
	21 & 23	Week 15	Fetal Development & Birth Systems
	28 & 30	Week 16	Assisted Reproduction/Contraception
May	5 & 7	Week 17	Exam or Grant Review