OVULATION & OVUM TRANSPORT IN MAMMALS

M.SC SEMESTER II PAPER 06

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INTRODUCTION

The process of setting free of mature ova from the ovaries is called ovulation. It occurs by different methods in different vertebrates.

Egg fertilization involves a complex sequence of events that starts with the release of a mature egg from the follicle, continues with the appearance of the two pronuclei after sperm entry, and is completed with the first mitotic division. Understanding the complexities of this process in mammals has been limited to a large extent by ethical constraints. However, with the advent of assisted reproductive technologies (ARTs), understanding of the various mechanisms involved in successful fertilization has been greatly enhanced.

EGG TRANSPORT

Egg transport refers to the movement of the oocyte from the moment of expulsion from the ovarian follicle to entry into the distal segment of the fallopian tube before fertilization takes place. Once fertilized in the ampullary segment of the fallopian tube, the embryo spends about 5 days traveling into the remaining anatomical oviductal districts and arrives into the uterine cavity at the blastocyst stage. For purposes of clarity and accuracy, the term "egg transport" covers post-ovulation and pre-fertilization stages (i.e. the haploid life span of the ovulated oocyte). A subsequent section provides details concerning transport of the fertilized diploid oocyte (i.e. zygote) and pre-implantation embryo.

The anatomy and physiology of the fallopian tube play an important role in egg transport and fertilization. The fallopian tube is a muscular tube with an average length of about 11–12 cm and is composed of four regions. The most distal portion is called the infundibulum, it is approximately 1 cm in length, and it includes the finger-like fimbria. The epithelial lining of the fimbria is densely ciliated and highly convoluted. This structure, along with the muscle-controlled movements of the fimbria, is thought to be important for capture of the cumulus-oocyte complex. The next portion of the oviduct is called the ampulla. This segment averages 5–8 cm in length. It is within this highly ciliated portion of the oviduct that fertilization

and early embryo development occur. The ampulla is most often also the site for ectopic implantation (ectopic pregnancy). The next region, approximately 2–3 cm in length, is the isthmus. Like the ampulla, it too is ciliated yet less densely so. The isthmus is thought to regulate sperm and embryo transport. The last segment of the fallopian tube is called the intramural segment; it is the link between the isthmus of the oviduct and uterine cavity.

The ciliated and non-ciliated cells of the fallopian tube undergo cyclic changes with the menstrual cycle similar to those occurring in the endometrium. Further, each portion of the fallopian tube appears to be preferentially regulated by hormones that cause a distinct regionalization of activities depending on the day in the female reproductive cycle. For example, in the early follicular phase (day 4), propulsive forces operate throughout the length of the fallopian tube in the direction of the uterus. At day 8 (mid-follicular phase), the ampulla has alternating propulsive forces towards and away from the uterus. At the time of ovulation (around cycle day 14), ipsilateral transport to the ovary increases with increasing follicular diameter. It has been observed that pregnancy rates after intercourse are higher in those women who demonstrate ipsilateral transport, as opposed to those who fail to show lateralization. The fallopian tube function is critical for the early stages of fertilization.

At the time of ovulation, the oocyte is surrounded by a mass of specialized granulosa cells called the cumulus oophorus. Together, the oocyte and granulosa cells are called the cumulus-oocyte complex (COC). The innermost cell layers of the cumulus immediately overlying the zona pellucida of the oocyte are called the coronal cells. After cumulus maturation, the same cells are called the corona radiata because of their "sunburst" appearance. These cells have processes that extend through the acellular glycoprotein matrix of the zona to contact the oocyte plasma membrane for a rich metabolic exchange of nutrients via the so-called transzonal projections. The cumulus of the mature COC is sticky and is thought to facilitate the adherence of the COC to the surface of the fimbriae once it is expelled from the follicle at ovulation.

The exact mechanism by which the COC is picked up and gains entry into the fallopian tube lumen is unknown. One possibility is that the fimbriated end of the ipsilateral fallopian tube sweeps over the ovary, picks up the COC, and draws it into the tubular lumen by muscular control. Paradoxically, women have become pregnant who were missing the fallopian tube on the side where ovulation occurred. Also, oocytes placed in the peritoneal cavity have been picked up by the fallopian tube and resulted in intrauterine pregnancies. This evidence suggests that

other forces help to facilitate oocyte pickup. Another possibility is that the rhythmic and unidirectional beating of cilia on the fimbriae – where the cilia have adhesive sites – and in the ampullary and isthmic regions of the fallopian tube, draw the COC into the lumen of the fallopian tube. Another possibility is that muscular contractions of the fallopian tube create negative pressure that helps to aspirate the COC from the surface of the ovary into the lumen. However, capping and suturing of the fimbriated end has failed to prevent pregnancy. More recently, researchers have reported that the uterus and fallopian tube appear to act as a peristaltic pump. The pumping frequency increases on the ipsilateral side, in the direction where ovulation will occur, and as the follicular diameter increases. A novel alternative to the aforementioned mechanisms for COC pickup is one involving mucus strand connections between fimbria and ovary that act as a tether between the two structures to facilitate fimbrial capture of the COC. The entire process of pickup and deposition of the COC into the lumen takes between 2 and 3 minutes after ovulation. Therefore, it would seem that at least several mechanisms are involved with COC pickup, the most important of which are ciliary beating, sweeping of the ovarian surface by the fimbria, and peristaltic pumping of the female tract.

FERTILIZATION

After ovulation, the fertilizable life span of the mammalian oocyte is estimated to be about 24 hours. In contrast, the fertilizable life span of the spermatozoon is around 72 hours. Sperm motility can persist for much longer and has been documented in vivo for up to 5 days, but fertilizing ability is lost before motility. Sperm deposited in the proximal vagina can be found in the fallopian tube within 5 minutes.

A number of sperm-related events must occur for successful fertilization. The first factor is that a sufficient number of mature, viable spermatozoa must be present in the ejaculate. Second, the morphology of the sperm must be such that the cervical mucus will allow passage into the uterus. Third, it is essential that a good percentage of the sperm have forwardly progressive motion to propel them through the cervical mucus into the uterine cavity and the fallopian tube for ultimate encounter with the COC. Fourth, sperm must undergo the acrosome reaction and hyperactivation during sperm transport into the female reproductive tract (vagina, uterus and tubes) to be enabled for cumulus cell penetration and zona pellucida binding.

The term capacitation derives from the observation that sperm must spend time in the female reproductive tract in order to acquire the capacity or ability to fertilize an oocyte. Sperm can also undergo capacitation in vitro when they are incubated in media containing serum albumin as well as energy substrates and electrolytes. Capacitation begins as sperm swim through the cervical mucus. Proteins absorbed in the plasma membrane are removed and sperm surface molecules are modified. An efflux of cholesterol from the sperm plasma membrane may be the initiating event for capacitation. The sperm plasma membrane and outer acrosomal membrane have increased permeability and fluidity as a result of these changes. The more permeable sperm plasma membrane allows for influx of calcium and bicarbonate resulting in activation of second messengers and initiation of signaling events. These unique changes that prepare the spermatozoon for fertilization have collectively been termed capacitation.

Some events that occur to induce capacitation are (1) an increase in membrane fluidity (2) a decrease in net surface charge; (3) an increase in oxidative processes and cyclic adenosine monophosphate (cAMP) production; (4) a decrease in the ratio of plasma membrane cholesterol to phospholipid; (5) expression of mannose binding sites as a consequence of cholesterol removal; (6) an increase in tyrosine phosphorylation; (7) an increase in reactive oxygen species; and (8) changes in sperm swimming patterns, termed hyperactivation. The hyperactive beat of the sperm flagellum is believed to help the sperm traverse the cumulus cell complex and bind to the zona pellucida. Successful capacitation of the sperm results in a hyperactivated spermatozoon, which is able to bind to the zona pellucida and is susceptible to acrosome reaction induction.

The acrosome reaction is an exocytotic process occurring in the sperm head that is essential for penetration of the zona pellucida and fertilization of the oocyte. The acrosome is a unique organelle, located in the anterior portion of the sperm head analogous to both a lysosome and a regulated secretory vesicle. One of the principal enzymes involved is a serine glycoproteinase called acrosin. It exists in a proenzyme form called proacrosin, which is converted to the active form acrosin by changes in acrosomal pH.

When sperm bind to the zona pellucida, intracellular calcium is low. The binding causes an opening of calcium channels and an influx of calcium and second messengers that result in the acrosome reaction. Other substances may also induce the acrosome reaction. For example, the addition of periovulatory follicular fluid or progesterone to capacitated spermatozoa stimulates an influx of calcium ions that is coincident with the acrosome reaction. Periovulatory follicular fluid contains

progesterone and it is thought that perhaps the progesterone stimulates calcium influx and the acrosome reaction. However, other acrosome reaction-stimulating factors (e.g. atrial natriuretic peptide) have also been detected in this complex fluid and may play a role in fertilization.

After a spermatozoon passes through the zona pellucida, it must contact, bind to, and fuse with the oocyte plasma membrane. As a result of the prior acrosome reaction, new sperm membrane proteins become exposed that are likely to prove integral for sperm-oocyte fusion. Data indicate that sperm-oocyte fusion is initiated by signal transduction processes that involve adhesion molecules on both sperm and oocyte plasma membranes that belong to the family of integrins. Integrins are a class of heterodimeric adhesion receptor molecules that participate in cell-to-cell and cell-to-substratum interactions, and they are present on essentially all mammalian cells. Integrins that recognize the Arg-Gly-Asp sequence (RGD) have been detected on the plasma membrane of oocytes. Fibronectin and vitronectin are glycoproteins that contain functional RGD sequences, and they are present on spermatozoa. When oligopeptides specifically designed to block fibronectin or vitronectin receptors were tested on human spermatozoa in a zona-free hamster oocyte assay, it was found that the peptide for blocking cell attachment to fibronectin was without effect but the other peptide, which blocks both fibronectin and vitronectin receptors, inhibited sperm-oocyte binding.

At some point during or after the fusion process, the oocyte is activated by the spermatozoon. Activation involves the resumption of meiosis through inactivation of metaphase promoting factor (MPF) which functions to arrest the oocyte in metaphase of the second meiotic division. Extrusion of the second polar body occurs and cortical granules are released into the perivitelline space. The cortical granules modify zona glycoproteins 2 and 3 on the inner aspect of the zona pellucida, resulting in a loss of their ability to stimulate the acrosome reaction and tight binding, so as to prevent polyspermy. This latter event occurs before or simultaneously with the resumption of meiosis. Failure of the oocyte to synthesize or release the cortical granules in a timely fashion results in polyspermic fertilization.

The first event after incorporation of the spermatozoon into the oocyte is the production of sperm-induced calcium (Ca^{2+}) transients. Calcium is the main intracellular signal responsible for the initiation of oocyte activation. These calcium fluxes occur in series and over time (termed “calcium oscillations”); when only a single transient is induced, either by chemical or mechanical stimulation, the oocyte fails to activate. The mechanism by which sperm induce calcium transients

is unknown, but there are data that support essentially two models for sperm-induced oocyte activation.

One proposed mechanism for sperm-induced oocyte activation is the binding of the spermatozoon to a receptor on the oolemma, which results in G-protein activation, activation of an amplifying enzyme, and generation of an intracellular second messenger within the oocyte. A second possible mechanism for sperm-induced oocyte activation can loosely be termed the "fusion hypothesis". In this model, at the time of sperm and oocyte membrane fusion a "latent" period ensues. During this latent period, a soluble sperm-derived factor diffuses from the sperm into the oocyte's cytoplasm and results in oocyte activation. To date, however, there are no published reports demonstrating that the extract from a single spermatozoon was able to activate an oocyte. Abnormalities in transcription, translation, or any other significant molecular process responsible for producing the oocyte-activating ligand/effector molecule during spermatogenesis or spermiogenesis will inhibit fertilization.

Progesterone secreted by the cumulus cells that surround the oocyte stimulates calcium signals that can control hyperactivation and the acrosomal reaction, however, the signaling mechanism has remained unclear.

As the sperm nucleus is undergoing oocyte-mediated decondensation, the sperm centrosome is orchestrating pronuclear mobilization, syngamy, and, ultimately, early cleavage. The sperm centrosome, with the assistance of maternal γ-tubulin, nucleates sperm astral microtubules and forms the mitotic spindle. The sperm aster, the name for the radial array of these microtubules, unites paternal and maternal pronuclei. At the time of fertilization, the sperm introduces the centrosome, which is the organizing center for microtubules. In doing so, it establishes the polarity and three-dimensional structure of the embryo. In humans, defects in microtubule organization are one cause of fertilization failures seen with in vitro fertilization and may explain fertilization failures that occur after intracytoplasmic sperm injection (ICSI).

EMBRYO TRANSPORT

As mentioned previously, fertilization occurs in the ampullary segment of the fallopian tube. Transit time of the zygote from the ampulla to the ampulla-isthmic junction is approximately 30 hours, after which the zygote remains in the isthmus another 30 hours before resuming transit through the isthmus. It is not until the 5th or 6th day after fertilization that the pre-implantation embryo arrives into the

uterine cavity. During the time frame from fertilization to deposition of the embryo in the uterus, the propulsive forces in the fallopian tube are towards the uterus.

The fallopian tube and its microenvironment are ideal for early embryo development.

In conclusion, information has been obtained in the past decade concerning the processes of mammalian fertilization and implantation.