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Question

1. **Discuss Ovulation**

**Ovulation** is the release of eggs from the ovaries. In women, this event occurs when the ovarian follicles rupture and release the secondary oocyte ovarian cells. After ovulation, during the luteal phase, the egg will be available to be fertilized by sperm. In addition, the uterine lining (endometrium) is thickened to be able to receive a fertilized egg. If no conception occurs, the uterine lining as well as blood will be shed during menstruation.

Ovulation occurs about midway through the menstrual cycle, after the follicular phase, and is followed by the luteal phase. Note that ovulation is characterized by a sharp spike in levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), resulting from the peak of estrogen levels during the follicular phase.

In humans, ovulation occurs about midway through the menstrual cycle, after the follicular phase. The few days surrounding ovulation (from approximately days 10 to 18 of a 28-day cycle), constitute the most fertile phase. The time from the beginning of the last menstrual period (LMP) until ovulation is, on average, 14.6 days, but with substantial variation among females and between cycles in any single female, with an overall 95% prediction interval of 8.2 to 20.5 days.

The process of ovulation is controlled by the hypothalamus of the brain and through the release of hormones secreted in the anterior lobe of the pituitary gland, luteinizing hormone (LH) and follicle-stimulating hormone (FSH. In the preovulatory phase of the menstrual cycle, the ovarian follicle will undergo a series of transformations called cumulus expansion, which is stimulated by FSH. After this is done, a hole called the stigma will form in the follicle, and the secondary oocyte will leave the follicle through this hole. Ovulation is triggered by a spike in the amount of FSH and LH released from the pituitary gland. During the luteal (post-ovulatory) phase, the secondary oocyte will travel through the fallopian tubes toward the uterus. If fertilized by a sperm, the fertilized secondary oocyte or ovum may implant there 6–12 days later.

**Follicular phase**

The follicular phase (or proliferative phase) is the phase of the menstrual cycle during which the ovarian follicles mature. The follicular phase lasts from the beginning of menstruation to the start of ovulation.

For ovulation to be successful, the ovum must be supported by the corona radiata and cumulus oophorous granulosa cells. The latter undergo a period of proliferation and mucification known as cumulus expansion. Mucification is the secretion of a hyaluronic acid-rich cocktail that disperses and gathers the cumulus cell network in a sticky matrix around the ovum. This network stays with the ovum after ovulation and has been shown to be necessary for fertilization.

An increase in cumulus cell number causes a concomitant increase in antrum fluid volume that can swell the follicle to over 20 mm in diameter. It forms a pronounced bulge at the surface of the ovary called the blister.

**Ovulation**

Estrogen levels peak towards the end of the follicular phase. This, by positive feedback, causes a surge in levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). This lasts from 24 to 36 hours, and results in the rupture of the ovarian follicles, causing the oocyte to be released from the ovary.

Through a signal transduction cascade initiated by LH, proteolytic enzymes are secreted by the follicle that degrade the follicular tissue at the site of the blister, forming a hole called the *stigma*. The secondary oocyte leaves the ruptured follicle and moves out into the peritoneal cavity through the stigma, where it is caught by the fimbriae at the end of the fallopian tube. After entering the fallopian tube, the oocyte is pushed along by cilia, beginning its journey toward the uterus.

By this time, the oocyte has completed meiosis I, yielding two cells: the larger secondary oocyte that contains all of the cytoplasmic material and a smaller, inactive first polar body. Meiosis II follows at once but will be arrested in the metaphase and will so remain until fertilization. The spindle apparatus of the second meiotic division appears at the time of ovulation. If no fertilization occurs, the oocyte will degenerate between 12 and 24 hours after ovulation. Approximately 1-2% of ovulations release more than one oocyte. This tendency increases with maternal age. Fertilization of two different oocytes by two different spermatozoa results in fraternal twins.

The mucous membrane of the uterus, termed the functionalis, has reached its maximum size, and so have the endometrial glands, although they are still non-secretory.

**Luteal phase**

The follicle proper has met the end of its lifespan. Without the oocyte, the follicle folds inward on itself, transforming into the corpus luteum (pl. corpora lutea), a steroidogenic cluster of cells that produces estrogen and progesterone. These hormones induce the endometrial glands to begin production of the proliferative endometrium and later into secretory endometrium, the site of embryonic growth if implantation occurs. The action of progesterone increases basal body temperature by one-quarter to one-half degree Celsius (one-half to one degree Fahrenheit). The corpus luteum continues this paracrine action for the remainder of the menstrual cycle, maintaining the endometrium, before disintegrating into scar tissue during menses.

**Disorders**

Disorders of ovulation are classified as menstrual disorders and include oligoovulation and anovulation:

* Oligoovulation is infrequent or irregular ovulation (usually defined as cycles of greater than 36 days or fewer than 8 cycles a year)
* Anovulation is absence of ovulation when it would be normally expected (in a post-menarchal, premenopausal female). Anovulation usually manifests itself as irregularity of menstrual periods, that is, unpredictable variability of intervals, duration, or bleeding. Anovulation can also cause cessation of periods (secondary amenorrhea) or excessive bleeding (dysfunctional uterine bleeding).

The World Health Organization (WHO) has developed the following classification of ovulatory disorders:

* WHO group I: Hypothalamic–pituitary-gonadal axis failure
* WHO group II: Hypothalamic–pituitary-gonadal axis dysfunction. WHO group II is the most common cause of ovulatory disorders, and the most common causative member is polycystic ovary syndrome (PCOS).
* WHO group III: Ovarian failure
* WHO group IV: Hyperprolactinemia

**Ovulation Induction**

Ovulation induction is a promising assisted reproductive technology for patients with conditions such as polycystic ovary syndrome (PCOS) and oligomenorrhea. It is also used in in vitro fertilization to make the follicles mature before egg retrieval. Usually, ovarian stimulation is used in conjunction with ovulation induction to stimulate the formation of multiple oocytes. Some sources include ovulation induction in the definition of *ovarian stimulation*.

A low dose of human chorionic gonadotropin (HCG) may be injected after completed ovarian stimulation. Ovulation will occur between 24–36 hours after the HCG injection.

By contrast, induced ovulation in some animal species occurs naturally, ovulation can be stimulated by coitus.

**Ovulation Suppression**

Combined hormonal contraceptives inhibit follicular development and prevent ovulation as a primary mechanism of action. The *ovulation-inhibiting dose* (OID) of an estrogen or progestogen refers to the dose required to consistently inhibit ovulation in women.

In assisted reproductive technology including in vitro fertilization, cycles where a transvaginal oocyte retrieval is planned generally necessitates ovulation suppression, because it is not practically feasible to collect oocytes after ovulation. For this purpose, ovulation can be suppressed by either a GnRH agonist or a GnRH antagonist, with different protocols depending on which substance is used.

1. **Differentiate between meiosis 1 and meiosis 2**

* First, in prophase 1(meiosis 1) chromatin condenses to form chromosomes which are paired to give homologous pairs. While prophase 2(meiosis 2) does not have any pairing.
* Secondly, in anaphase 1(meiosis 1), the pair breaks and one chromosome each moves to the opposite side of the pole. While anaphase 2(meiosis 2) has chromosomes broken into sister chromatids that move to opposite poles.
* Thirdly, in meiosis 1 a diploid cell gives two haploid cells. while in meiosis 2 two haploid cells divide to give four haploid cells.

1. **Discuss the stages involved in fertilization**

The Four Steps of Fertilization:

**Step I. Preparation of the Sperm.**

Ejaculated sperm are not ready to fertilize an egg when they enter the vagina. In response to the dilution of semen in the vagina, they undergo several changes, which are collectively known as **capacitation**.

1.Intracellular Ca++ levels increase.

2.Spermatic motility is activated and tails change beat

frequency.

3.Sperm cell surface antigens are lost. The loss of

these proteins renders the sperm more receptive to

binding to the egg.

**Step II. Sperm-Egg Binding**

Because of the availability of gametes, the process of sperm-egg binding was first studied and understood in invertebrates. In sea urchins, the sperm head binds directly to the egg outer surface and this triggers the acrosome reaction.

The acrosomal contents are released and there is a balanced Na+ influx and H+ efflux, causing an increase in pH. The increased pH triggers the dissociation of the profilactin complex (actin and profilin) and the released actin monomers polymerize to form a filament called the acrosomal process. This acrosomal process penetrates the egg coatings to allow fusion of the sperm and egg plasma membranes. In sea urchins then, the sperm literally skewers the egg.

In humans the process of sperm-egg binding is not so simple. The complicating factor is the thick zona pellucida, which keeps sperm from binding close to the egg plasma membrane.

**Sperm receptor on egg.**

Dr. Paul Wassarman used a **competition assay** to isolate and identify the factor in the zona pellucida that was involved in sperm egg binding. Dr. Wassarman incubate sperm with zona pellucida glycoproteins (ZPGPs) he had isolated from unfertilized and fertilized eggs. He found that sperm preincubated with ZPGPs from unfertilized eggs were not able to fertilize eggs. Yet, when he preincubated sperm with ZPGPs isolated from fertilized eggs, which are known not to bind sperm, the sperm could still fertilize eggs. This showed that the isolated ZPGPs from unfertilized eggs contain a receptor for the sperm and that this receptor is modified after fertilization.

In follow up experiments, Dr. Wassarman purified ZPGP I, ZPGP II and ZPGP III and showed that only ZPGP III could prevent sperm binding to eggs showing that ZPGP III is the sperm receptor. By treating ZPGP III with agents that selectively hydrolyzed protein (trypsin), N-linked glycoproteins (specific glycohydrolase) and O-linked glycoproteins (weak base), Dr. Wassarman showed that the part of ZPGP III that was responsible for sperm binding was the O-linked oligosaccharide.

**Egg receptor on sperm.**

What sperm component is binding to the ZPGP III? Dr. Barry Shur was studying a Golgi enzyme known as **galactosyl transferase**. This enzyme catalyzes the addition of galactosyl residues from a donor nucleotide sugar, UDP-galactose, to the terminal end of O-linked oligosaccharides. As in all enzymatic reactions, there are two stages in catalysis:

1.The enzyme binds the substrates (in this case UDP-gal and O-linked oligosaccharide).

2.The enzyme catalyzes the reaction and releases the products (in this case, UDP and the modified O- linked oligosaccharide with galacosyl residues on its ends).

It is important to understand that if one of the substrates is not present, the enzyme may be able to bind the available substrate, but will not be able to catalyze the reaction. This is important in sperm binding.

Dr. Shur found that sperm, which have no Golgi apparatus, have galactosyl transferase on the surface of their plasma membrane. When sperm are ejaculated, they have oligosaccharides bound to the galactosyl transferase. During capacitation, these coating glycoproteins are removed, allowing the galactosyl transferase, to bind to other carbohydrates it may encounter, such as those attached to ZPGP III. The sperm that do encounter the egg and its zona pellucida, bind ZPGP III through their galactosyl transferases. At this point, UDP-gal would normally bind to its site on galactosyl transferase, galactose residue would be transferred to the oligosaccharide and the modified oligosaccharide would be released. However, there is no high energy UDP-galactose in the extracellular fluid surrounding the egg so catalysis does not occur and the sperm remains tightly bound to the egg zona pellucida.

Many studies support a role for galactosyl transferase as the sperm protein involved in sperm-egg binding, however, other proteins may be involved. A recent genetic knockout of galactosyl transferase in mice yielded mice that were completely fertile and showed normal sperm-egg binding.

**Acrosome reaction**.

As a result of irreversible binding of the sperm to the egg, the zona pellucida triggers the acrosome reaction. The outer plasma membrane of the acrosome fuses at multiple sites with the plasma membrane and the contents of the acrosome are released. Two of the important components are **acrosin**, a serine protease, and **N-acetylglucoaminidase**. Acrosin bores a hole in the zona pellucida so that the sperm can reach the egg itself. N-acetylglucoaminidase hydrolyzes the O-linked oligosaccharides in ZPGP III to allow the sperm to detach. As a result of the membrane fusion, a new surface is exposed on the sperm (the inner acrosomal membrane) and this is thought to contain new binding sites for ZPGP II.

**STEP III. Sperm-Egg Fusion.**

For many years the process by which the plasma membrane of the sperm and egg fused was a black box. Recent studies by Drs. Judith White, Diana Miles, and Paul Primakoff andtheir colleagues, have now shed light on this process. Milesand Primakoff made an antibody to PH-30, a heterodimericsperm membrane protein comprised of α and β subunits, and showed that this antibody blocked fertilization but did not block binding of sperm to eggs stripped of their zona pellucida. This suggested that PH-30 was involved in sperm and egg fusion and it was given the name **fertilin**.

Cloning and sequencing of fertilin revealed that the α-subunit had a hydrophobic domain that resembled those on viral proteins that are known to be involved in membrane fusion. The β-subunit had a **disintegrin** domain. Disintegrins were first discovered in snake venom and act as competing ligands for integrins (for example, snake venom disintegrins will block platelet aggregation mediated by integrins). Both subunits had metalloprotease domains. Fertilin was one of the first proteins of a family of proteins known as ADAMs proteins (for A Disintegrin And Metalloprotease containing protein) that are involved in cell-cell recognition and cell fusion events. Although the mechanism for how fertilin causes sperm-egg membrane fusion is not known, studies have supported its role in membrane fusion. For example, a peptide corresponding to the viral fusion peptide of α-fertilin is capable of fusing model membrane vesicles and the disintegrin domain of β-fertilin will block sperm-egg fusion. The egg integrin involved in sperm-egg fusion (the receptor for the β-subunit

disintegrin) is known to be α6β1. Once the sperm fuses with the egg, the beating of the

tail stops immediately. The sperm instead is drawn into the egg by elongation and fusion of the egg’s microvilli. As a result, the sperm nucleus and other organelles are incorporated into the egg cytoplasm. The sperm nucleus undergoes a series of

changes, including chromatin decondensation and formation of a new nuclear envelope, to form a male **pronucleus**. The male pronucleus uses microtubules to migrate to the center of the cell, where it fuses with the female pronucleus to reconstitute a diploid nucleus. Other sperm organelles (e.g. Mitochondria) persist during early cleavage stages of the embryo and it is conjectured that they may play a

role in development.

**STEP IV. Activation - The Egg’s Response.**

The immediate events after fertilization include the egg’s effort to prevent polyspermy.

Polyspermy refers to the fertilization of the egg by more than one sperm, resulting in zygotes with greater than a diploid amount of DNA. This causes early embryonic defects and arrest of development.

After sperm-egg fusion, the egg mounts the **cortical reaction** to prevent polyspermy. In all eggs, residing just under the plasma membrane there are membrane bound vesicles known as **cortical granules**. When a single sperm penetrates the egg, the cortical granules adjacent to the site are triggered to fuse with the plasma membrane, exocytosing their contents into the perivitelline space (the space between the plasma membrane and the zona pellucida). The cortical reaction is propagated over the

surface of the egg by a wave of Ca++ . This was shown by the aequorin experiment in which the photoprotein aequorin phosphoresced in a wave from the site of sperm penetration of the egg.

As a result of the cortical reaction, two important enzymes are released into the perivitelline space:

1. Ovoperoxidase: In sea urchins, ovoperoxidase catalyzes the crosslinking of tyrosine residues in the extracellular matrix. This makes the extracellular matrix tough and insoluble (analogous to the tanning of leather) and a physical barrier is formed which prevents other sperm from fertilizing the egg. In mammals, ovoperoxidase does not catalyze tyrosine cross-linking to the point of insolubility. In mammals, its major effect is thought to be as a spermicial agent.

2. Hydrolase. Remember Wassarman’s result showing that zona pellucida from fertilized eggs was incapable of blocking fertilization? Another cortical granule that is released is a specific hydrolase, which degrades O-linked oligosaccharides on ZPGP III. This renders the zona pellucida incapable of binding additional sperm, thus preventing polyspermy. Activation of the egg also includes the initiation of development of the new zygote. Protein synthesis and other metabolic processes are upregulated to provide for the developing embryo.

1. **Differentiate between Monozygotic twins and Dizygotic twins**

Difference Between Monozygotic and Dizygotic Twins

**Development**

Monozygotic Twins: Monozygotic twins are developed by the splitting of a fertilized embryo into two.

Dizygotic Twins: Dizygotic twins are developed by two separate fertilization events occurring at the same time.

**Causes**

Monozygotic Twins: The cause for monozygotic twins is not known.

Dizygotic Twins: Dizygotic twins is caused either by IVF, certain fertility drugs or hereditary predisposition due to

the hyper­ovulation.

**Called as**

Monozygotic Twins: Monozygotic twins are called identical twins.

Dizygotic Twins: Dizygotic twins are called fraternal twins.

**Genetic Code**

Monozygotic Twins: The genetic codes of the monozygotic twins are nearly identical.

Dizygotic Twins: The genetic code of the dizygotic twins are same as any other sibling

**Gender of Twins**

Monozygotic Twins: The gender of monozygotic twins are same.

Dizygotic Twins: The gender of dizygotic twins are different.

**Blood Type**

Monozygotic Twins: The blood type of monozygotic twins are the same.

Dizygotic Twins: Dizygotic twins may have different blood types.

**Appearance**

Monozygotic Twins: Monozygotic twins are extremely similar. But, they may vary depending on the environmental

factors.

Dizygotic Twins: The appearance of dizygotic twins is similar as any other sibling.

**Likelihood**

Monozygotic Twins: The likelihood of the monozygotic twins is uniform around the world.

Dizygotic Twins: The likelihood of the dizygotic twins varies by country.

**Occurrence**

Monozygotic Twins: One­third of the twins in the world are monozygotic twins.

Dizygotic Twins: Two­thirds of the twins in the world are dizygotic twins.

**Hereditary**

Monozygotic Twins: Monozygotic twins are not hereditary.

Dizygotic Twins: Dizygotic twins are hereditary.

**Risk of Twin­to­Twin Transfusion Syndrome (TTTS)**

Monozygotic Twins: Monozygotic twins bear high risk for TTTS.

Dizygotic Twins: Dizygotic twins bear a low risk for TTTS compared to monozygotic twins