Reproduction – coitus, fertilization and implantation

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Transport of spermatozoa in the male tract

Sperm undergo ***maturation*.**

- Seminiferous tubules → rete testis → vasa efferentia → epididymis → vas deferens.
- The fluid of the seminiferous tubules is secreted constantly by the Sertoli cells. In the *rete testis*, changes in ionic and small molecule composition occur by diffusion.
- In the vasa efferentia and **epididymis,** major changes occur in the fluid and sperm.
  (1) The sperm are concentrated 100-fold by fluid absorption (from 50×10^6/ml to 50×10^8/ml).
  (2) The epididymis adds some secretory products and also modifies the glycoprotein coat on the surface of the sperm. Instead of a saturated lipid membrane, they end up with a more fluid membrane structure that is stabilised by adsorbed glycoproteins.
  (3) Before this stage, spermatozoa were incapable of movement and of attaching to and fertilizing eggs. After the epididymis, they can swim effectively. An increase in cAMP in the tail is involved.
  (4) The sperm become capable of metabolising fructose.
- Note that the anatomical divisions of the epididymis (caput, corpus, cauda) are not consistently related to the histology (initial segment – absorptive; middle segment – secretory; terminal segment – storage) across species.
- The epididymis is a target organ for testosterone and depends on it. It bears intracellular testosterone receptors and contains 5α-reductase to make the more active form, DHT. Some testosterone comes to it in the rete testis fluid, carried on ABP.
- The **vas deferens** is muscular and can propel the sperm along (there is hardly any fluid for them to swim in).[1] The vas deference serves as a storage reservoir of spermatozoa. In the absence of ejaculation, spermatozoa dribble through the terminal *ampulla* of the vas deferens into the urethra and are washed away upon urination.

- **Semen = spermatozoa + seminal fluid.** Seminal fluid is not essential for effective sperm function, but is important as a transport medium and may supply nutritional and protective factors. Boars make ~0.5l; humans make ~3ml. Semen volume is 10% from the vas deferens, 60% from the seminal vesicles, 30% from the prostate and small amounts from the mucous glands (esp. the bulbourethral glands).
- The **seminal vesicles** secrete a mucoid material containing an abundance of *fructose* and other nutrients, *prostaglandins* and *fibrinogen*. They empty their contents into the ejaculatory duct shortly after the vas deferens has emptied the sperm. This adds greatly to the ejaculated volume and washes the sperm out of the ejaculatory duct. The function of the nutrients is obvious; the prostaglandins (1) react with cervical mucus to make it more receptive; (2) may cause reverse peristaltic contractions in the uterus and oviducts (a few sperm reach the upper end of the Fallopian tubes within 5 minutes).
- The **prostate** secretes a thin, milky, alkaline fluid containing *citrate*, *calcium*, *acid phosphate*, a *clotting enzyme* and a *profibrinolysin.* Its capsule contracts simultaneously with the vas deferens to expel this fluid during ejaculation. The alkalinity may be very important for successful fertilization: the fluid of the vas deferens is quite acidic with the end products of sperm metabolism, as is the vaginal fluid (pH 3.5–4.0), while sperm are not optimally motile until the pH rises to 6–6.5. The clotting enzyme acts on fibrinogen to form a weak coagulum that holds the semen in the vagina, and then lyses over then next 15–30 min thanks to fibrinolysin (formed from the profibrinolysin). As this happens the sperm become highly mobile.
- Semen also contains reducing agents (including ascorbate); sperm are made in a low pO_2 environment and are then exposed to oxygen; they are sensitive to oxidation.
- Sperm can live for weeks in the male (though in practice they spend 4–5 days there) but once they are ejaculated their maximal lifespan is 24–48h at body temperature.

[1] Ligation of the vasa efferentia causes enormous damming up of fluid, but ligation of the vas deferens (as in vasectomy) does not. Spermatozoa do build up, though; they must be removed by phagocytosis or leakage.

Coitus

Pronunciation: *co*-it-us, not *coy*tus.

- Has a social as well as a sexual function in higher primates; obviously humans (menstruation is concealed and the female is receptive throughout the cycle) but archetypally bonobo chimpanzees.
- **EPOR model** in humans (Masters & Johnson).
    1. **Excitement.** Psychogenic or somatogenic stimuli cause sexual arousal.
    2. **Plateau.** Arousal is intensified; if stimulation is sufficient and prolonged, orgasm occurs.
    3. **Orgasm.** Brief moment of involuntary climax; intense pleasure; often myotonia.
    4. **Resolution.** Sexual arousal fades. Pelvic haemodynamics return to normal. Occurs rapidly (minutes) following orgasm but may take hours otherwise.
- This model applies to *both sexes.* In the male, there is an *absolute refractory period* following orgasm in which sexual re-arousal and orgasm is impossible; its duration varies with age (young=quick), and psychological factors including novelty of partner/context.[2]

The male.

- *Erection* results from tactile stimulation the penis and adjacent perineum. The reflex involves the internal pudendal nerves (afferent) and the parasympathetic outflow from S2–4 (efferent).[3] Psychogenic stimuli (e.g. visual cues) can also cause erection so there is descending control.
- Erection is caused by relaxation of the smooth muscle of the dorsal artery and the arteries to the corpus cavernosum. The arteries dilate, allowing an inflow of blood. Arterio-venous shunts are closed to prevent drainage and the SM of the cavernosum relaxes, decreasing resistance to the increase in blood volume there. Venous 'bleed' valves close (and the veins are compressed by the increased pressure). Low volume, low pressure → high volume, high pressure. The corpus spongiosum does not increase in turgor as much as the c.c., so compression of the urethra is avoided.
- Signalling pathways are still not certain. Autonomic NS involves NA and ACh. Injection of VIP causes erection, but may ultimately end up as a nitric oxide (NO) signal (enter Viagra).
- The testes are drawn reflexively towards the perineum and the dartos muscle contracts the scrotum. Testicular volume may increase by 50% due to vasocongestion.
- Further stimulation leads to *emission*, in which the contents of the vas deferens, prostate and seminal vesicles are expelled into the urethra.[4] This is followed by *ejaculation*, in which semen is expelled from the posterior urethra (urethral smooth muscle + bulbocavernosus + ischiocavernosus). Retrograde ejaculation into the bladder is prevented by contraction of the vesical urethral sphincter.
- Other changes of sexual arousal – nipple erection, ↑HR, ↑BP, skin rashes immediately prior to ejaculation, muscle spasms etc.

The female.

- Psychogenic stimulation, stimulation of the vaginal walls and particularly the clitoris leads to genital changes very similar to the male. Vasocongestion of genitalia, including clitoral erection. Other effects are also similar, though timecourse differs from the male (i.e. longer). Subjective descriptions of orgasms are very similar in men and women.
- Vaginal lubrication is by *transudation* of fluid through the vaginal wall.
- The vagina increases in width and length and the uterus elevates, lifting the cervical os to cause *tenting* of the vagina.
- At orgasm, vaginal and uterine contractions occur. The cervix may be actively dipped into the pool of semen by these contractions.
- Behavioural differences in sexual excitability probably reflect differences in reproductive strategy (see Ridley). Orgasm following coitus occurs in 100% of normal men but surveys suggest 30–50% of women.

[2] The **Coolidge effect,** named after the US President. The story goes that the Coolidges were on a tour of a chicken farm and Mrs Coolidge saw a cockerel mounting one of the hens. "How often does he do that?" she asked. "Oh, a good ten times a day, ma'am," replied the farmer. "Please tell that to the President," said Mrs Coolidge. On being appraised of this fact, the President asked "Always the same hen?" "Oh, no, Mr President, a different hen every time," came the reply, to which he responded "Tell that to Mrs Coolidge."

[3] **P**oint and **S**hoot – parasympathetics for erection, sympathetics for ejaculation. This is oversimplified but useful.

[4] Sympathetic NS. Alpha-blockers lead to 'dry orgasms'.

Transport of spermatozoa in the female tract

Sperm undergo ***capacitation*** and ***activation.***

- The ovum has halted in the ampullary–isthmic junction of the oviduct. Fertilization occurs here.
- The **cervix** is a physical and a physiological filter for sperm. It is in the female's interests to select high-quality sperm; only the best shall pass?
- Ejaculated sperm are poor at – or at least slow to – fertilize eggs *in vitro*. Sperm recovered from the oviduct a few hours after coitus are capable of immediate fertilization; they have undergone **capacitation** in the female tract. Capacitation involves the stripping off of the sperm's glycoprotein coat, acquired in the epididymis. An oestrogen-primed uterus is an optimal medium for this. Several factors probably contribute: the high ionic strength and the proteolytic enzymes present are important. These conditions elute the glycoproteins. This leads to increased membrane fluidity at the head and tail, and increases the calcium conductance.
- The sperm are still not fully capable of fertilization. The final change is **activation,** which is a Ca^{2+}-dependent event and involves three changes.
    1. The **acrosome reaction.** The acrosome swells and its membrane fuses with the plasma membrane at several points. This can release the contents of the acrosome.
    2. Tail movement. While ejaculated sperm move by undulating their flagella, capacitated sperm move with a powerful whiplash motion.
    3. The membrane overlying the middle (equatorial region) and the posterior half of the spermatozoal head changes. The change allows the membrane to fuse with the surface of the egg (it becomes 'fusible').

Fertilization

- After the acrosome reaction, the sperm will live for 15–30 minutes, so it needs to be near the egg when this happens. The exocytosis of the acrosome contents depends on a further internal rise in both Ca^{2+} and cAMP. The egg is surrounded by the cumulus cells, which act like a sponge and carry progesterone. *Progesterone can elicit the acrosome reaction.*
- (The method of depositing a reservoir of sperm in the cervix and then letting them trickle towards the egg where they are activated may be a mechanism of increasing fertility. You don't want them all dying straight away if the egg isn't there yet.)
- The acrosome reaction releases **hyaluronidase.** This can digest the intercellular matrix of hyaluronic acid holding the cumulus mass to the egg. The cumulus falls off. Some sperm may be sacrificed getting through the cumulus so others can make it further.
- The sperm is now next to the **zona pellucida,** which contains glycoproteins ZP1, ZP2 and ZP3.
- **ZP3** binds the apical membrane of sperm that have *not* undergone the acrosome reaction. This binding is **species-specific;** it is the only species-specific binding in the whole process. ZP3 *elicits* the acrosome reaction.
- **ZP2** binds the *inner* acrosomal membrane. This allows sperm to attach and digest a pathway through the ZP using the proteolytic enzyme **acrosin.** The hyper-activated tail pushes it forwards.
- The sperm enters the **subzonal (perivitelline) space,** next to the oolemma. Microvilli on the surface of the egg envelop the sperm head, which lies on its side and **fuses** at its mid- (equatorial) region, which is next to its nucleus. Sperm can only fuse if they have undergone the acrosome reaction. They can fuse with the egg anywhere except over the meiotic metaphase spindle.
- Fusion triggers *cessation of tail movement.* From entry into the cumulus mass to fusion takes 10–20 minutes.
- The oocyte is now fertilized and is a **zygote.** It faces two immediate problems: (1) stop more sperm coming in – the *block to polyspermy* – as that would cause *androgenetic triploidy/polyploidy*, and (2) complete its second meiotic division, to prevent *gynogenetic* triploidy.
- Fusion triggers a **$\uparrow Ca^{2+}$ wave** spreading over the egg. A secondary series of Ca^{2+} spikes occurs every 5–10 minutes for several hours and is due to release and resequestration of Ca^{2+} from internal stores. These stores are sensitive to IP_3 and Ca^{2+} itself (positive feedback). Mechanism unclear – currently favoured idea is that a G-protein-coupled receptor detects the sperm and causes IP_3 formation. **Ca^{2+} has two roles:**
    1. It causes exocytosis of vesicles ("cortical granules"). These vesicles contain enzymes which target the ZP.
        (a) a proteolytic enzyme cleaves ZP2 $\Rightarrow$ no more sperm can bind to it
        (b) hexosaminidase β digests the galactose-rich oligosaccharide chain on ZP3 $\Rightarrow$ no more acrosome reactions induced
        (c) an enzyme cross-links ZP2 to ZP3 $\Rightarrow$ renders ZP indigestible to acrosomal contents $\Rightarrow$ no more sperm can get through (any sperm halfway through get stuck)
    2. Meiosis is reactivated. The egg was arrested in 2nd meiotic metaphase. Now the *second polar body* forms (with one set of chromosomes in it) and is jettisoned. This is the first time the female gamete is haploid! Meiosis completes.[5]

[5] A protein called maturation promotion factor (MPF), unique to the egg, has stabilized the meiotic spindle thus far. In turn it is stabilized by cytostatic factor (CSF). Ca^{2+} destroys CSF, destabilizing MPF and the spindle, and off we go to interphase. Anyway, enough of this molecular stuff.

- Both problems solved!
- The reason it's so hard for sperm to get to the egg is because only one must be allowed to do it.
- If the spindle fails its task, chromosomes come adrift and monosomy and trisomy can result.[6] If the oocyte is kept waiting around for a few days before fertilization, p(abnormalities of somy/ploidy) increases. Maybe this accounts for the increases in trisomy in older women (eggs've been hanging around for years! ☺). The optimum is a freshly ovulated egg from a young woman.

- Now the **spermatozoal chromosomes** are 'unpacked' and the two **pronuclei** migrate to the centre of the egg. This is a zygote – the chromosomes are not mingled.
- At the end of the first cell cycle, the pronuclei break down and the chromosomes mingle to form a diploid nucleus – the process of **syngamy**. This is now an **embryo.** The chromosomes form up on the equator and undergo mitosis. They will not be transcriptionally active until the 4-cell stage.

Implantation

- As cell cleavage is occurring, the embryo moves towards the uterus (it's wafted there by the cilia of the oviduct and the intramural sphincter opens; this is dependent solely on steroid hormones). It arrives just as the blastocyst is about to form – at the 4½–5½ day stage.
- The blastocyst grows (5½–10½ d).
- Luteal regression will cause loss of the embryo. Probably 50–80% of embryos are lost this way.
- Human embryos make a **luteotrophic signal.** They put an enormous effort into making **human chorionic gonadotrophin (hCG),** which resembles LH.[7]

- Before attachment, embryonic nutrition is obtained histiotrophically from uterine secretions.
- In humans, attachment occurs at 7 days. The ZP is shed – in the human, the blastocyst digests a hole and 'hatches' from the ZP.
- The size of the attachment (⇒placental structure) and the intimacy of tissue interaction depends on the species. Humans have a small, discrete placenta, but the embryo **invades** the endometrium and is enveloped.[8] It erodes maternal tissue, including blood vessels, creating lakes of blood in which to dip its placental 'fingers'. The maternal response is called **decidualization.** (It probably prevents the embryo invading too far!)
- Decidualization requires a uterus exposed to high progesterone levels (to maintain secretion/state of endometrium) plus a secondary oestrogen exposure (changes the secreted material; alters the surface epithelium so it can respond to the embryo[9]).

- Up to the primitive streak stage, **MZ twinning** can occur. (Dizygotic twins – two eggs, two sperm. Monozygotic twins – one egg, one sperm, splits. If it splits at the 2-cell stage, you get two blastocysts; at the ICM stage you get two amnions and one placenta; still later, you get one amnion and one placenta.)
- 0.1% of the gastrulated conceptus becomes the fetus. The rest… nutritional support systems.

Know this:

trophectoderm	→	chorionic ectoderm (cyto- & syncytiotrophoblast)	→	chorion & placenta (together with some extra-embryonic mesoderm)	
inner cell mass (ICM)	→	extra-embryonic	ectoderm	} amnion	
			mesoderm	} }	
			endoderm	} yolk sac & allantois	
		embryonic	ectoderm	}	
			mesoderm	} embryo / fetus	
			endoderm	}	

[6] Triploidy = 3 of all chromosomes. Trisomy = 3 of one chromosome.
[7] Non-primate embryos make trophoblastic interferon (IFN τ) which acts on the endometrium to stop production of the luteolytic signal $PGF_{2\alpha}$.
[8] Other animals: a close apposition of surface epithelium, but no invasion.
[9] Heparin-binding epidermal growth factor (HBEGF) is made locally. The blastocyst has receptors for this and is stimulated to hatch and attach.