

Reproduction – contraception and assisted conception

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Contraception

Effectiveness measured as *pregnancies per hundred woman-years (HWY)*, e.g. if 1200 couples had regular sex for one month, how many pregnancies would there be. The following data is *approximate*.

Method	Failure rate
Tubal ligation / vasectomy	0.005–0.04
Combined oral contraceptive (Pill)	0.005–0.56
IUCD (coil)	0.5–3.5
Continuous progestagen	1.5–2.3
Diaphragm/condom + cream	
<i>All users</i>	4.0–7.0
<i>Highly motivated</i>	1.5–3.0
Periodic abstinence	
<i>All users</i>	10.0–30.0
<i>Highly motivated</i>	2.5–5.0
Coitus interruptus	30.0–40.0
Vaginal foam/cream	30.0–40.0
Post-coital douche	45.0
Prolonged breastfeeding	45.0

UK usage by sexually active, fertile women not trying for pregnancy is – sterilization (M+F; 26%), Pill (25%), barrier/‘natural’ methods (16%), IUCD (5%), none (20%).

Many methods have an obvious mechanism. But make sure you know...

- disease protection is a totally different matter! (“double Dutch” = Pill for contraception + condom against disease)
- condoms rely on spermicide for these rates
- others, e.g. female condom, vaginal sponge with spermicide, progestagen injections and implants
- **intra-uterine contraceptive device (IUCD)** is made of copper; this induces a sterile inflammatory reaction in the uterus and oviducts and is toxic to sperm. A new version (Mirena®) releases progesterone locally, lasts 5y and is effective as a contraceptive but isn’t licensed for this use.
- **combined oral contraceptive (COC)** is ethinyl oestradiol plus a progestagen. Oestrogen suppresses FSH/LH secretion → anovulatory state. Progestagen further suppresses LH secretion, renders the cervix impenetrable (and alters the endometrium). This mimics pregnancy! A progesterone-withdrawal period is built into the dosing system, *not* because it’s needed but because users want periods to know they’re not pregnant. “Double-packing” is safe.
- **progesterone-only Pill (POP, mini-Pill)** may be used if there are contraindications to COC but is less effective.
- **post-coital contraception** (morning after pill) – simply a high-dose Pill. Can also use mifepristone (RU-486), a progesterone antagonist also used for medical abortion; in addition, can fit a coil up to 5 days after intercourse (well, it works for longer, but then it’d be an illegal method of abortion).

Infertility

- Defined clinically as the inability to conceive in one year (of regular sex) – but note that 80–90% of normal couples conceive in 1y [so 10–20% would be called infertile!], rising to 95% after 2y. About a 10–15% chance of conceiving in any one month.
- **Anovulatory infertility** may be due to hypogonadotrophic hypogonadism (insufficient LH/FSH), polycystic ovary syndrome, hyperprolactinaemia (may be caused by stress!), weight-related (↑/↓) or premature ovarian failure.
- **Tubal disease** may be due to adhesions, tubal blockage following salpingitis (*salpinx* = oviduct) etc.
- **Uterine factors** include intra-uterine adhesions.
- **Cervical mucus ‘hostility’**, which is probably an immunological reaction to sperm.
- **Male infertility** may be due to
 1. defects in spermatogenesis – oligospermia (<20 million sperm/ml), azoospermia (no sperm whatsoever) or sperm abnormalities including asthenospermia (lack of movement). A host of causes (Klinefelter’s, cryptorchidism, varicocele, radiation, drugs, stress, infections, high temperature...).
 2. ductal defects
 3. semen factors, including retrograde ejaculation
 4. coital factors, including impotence.
- **Unexplained.**

Assisted conception

- **Treat cause** if possible. Tubal damage may be helped by surgery.
 - If male infertility is the problem, **artificial insemination** (by donor – AID / by husband – AIH) may work, though many couples dislike the idea of AID.
 - **Impotence. Viagra™** is sildenafil citrate. It inhibits a phosphodiesterase (PDE5) responsible for cGMP breakdown. Recall that nitric oxide is released in the corpus cavernosum during sexual stimulation and activates guanylate cyclase to increase cGMP. This produces smooth muscle relaxation in the corpus cavernosum and allows inflow of blood. Sildenafil potentiates the effect of NO but has no effect in the absence of sexual stimulation. (Better than penile injections!)
 - **In vitro fertilization** may be useful otherwise. £££. Sample essay below. Note that superovulation techniques greatly enhance the likelihood of multiple pregnancies – when you hear about quads in the news, you can bet that IVF techniques caused it.
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SAMPLE ESSAY. “Detail the difficulties of inducing in vitro fertilisation.”

Introduction to IVF

Although human reproductive efficiency is normally very high, a large proportion of human couples are estimated to be infertile (~20% in the UK). A number of *in vitro* techniques are now available to assist reproduction when natural conception fails. These methods are possible due to the development of defined culture media and modern tissue culture techniques, which enable the maintenance of human gametes and cleavage-stage embryos *in vitro*; micromanipulation techniques, which are used to manipulate oocytes and embryos; and freezing techniques allowing the storage of gametes and embryos. The first ‘test-tube baby’ was born in 1978, and the practice of IVF is now routine and safe to the couple concerned, although the success rate is very variable.

The procedure of ***in vitro* fertilization (IVF) and embryo transfer** (implantation in the uterus) is widely used when the oviducts are scarred, as commonly results from pelvic infections. The scarring either prevents the sperm from reaching the ampulla or prevents the fertilized oocyte from reaching the uterus.

Obtaining oocytes

The woman’s ovaries are first induced to **superovulate** (develop multiple mature follicles) by hormonal administration: usually human menopausal gonadotrophin and FSH, sometimes with clomiphene citrate. Another regime is to use a short protocol of buserelone acetate and human menopausal gonadotrophin, followed by an injection of hCG shortly before follicle puncture.

Maturing oocytes are then harvested from the follicles, usually by aspiration through a laparoscope. The laparoscope can be inserted through a small puncture in the umbilical region. Alternatively a needle can be inserted via the vagina and guided to the ovary using ultrasonography. Once harvested, the oocytes are placed in culture medium in a test tube or Petri dish and allowed to mature to the second meiotic metaphase.¹ Typically the oocytes are stripped of cumulus cells before fertilization is attempted, by incubation in Earle’s medium with hyaluronidase.

Obtaining spermatozoa; capacitation

The changes spermatozoa undergo to gain full fertilizing capacity are termed *maturation* in the male tract, and *capacitation* and *activation* in the female tract. Spermatozoa are usually obtained non-invasively from the male by masturbation and hence will have matured. Capacitation involves the stripping off of the spermatozoal glycoprotein coat acquired in the epididymis and seminal plasma. There is no single ‘capacitating factor’ but the most critical features *in vivo* are the proteolytic enzymes and high ionic strength fluid provided by the oestrogen-dominated uterus. These elute the glycoproteins. Suitable culture media can mimic this successfully. Activation is not fully understood at a molecular level but is Ca²⁺ and cAMP dependent. There may be a specific activation factor *in vivo* (from follicular fluid or the zona pellucida) but one is not required *in vitro*. The three classes of change that occur are the acrosome reaction (exposing the contents of the acrosomal vesicle to the exterior), the appearance of whiplashing beats of the tail, and a change in the surface membrane overlying part of the head enabling it to fuse with the egg.

From patients with agenesis or obstruction of the vas deferens spermatozoa may be obtained by **microsurgical epididymal spermatozoa aspiration (MESA)**. Although pregnancies have been achieved after MESA it is generally accepted that the fertilizing capacity of spermatozoa is reduced.

If spermatozoa cannot be obtained from the epididymis success may be achieved with testicular spermatozoa. Following testis biopsy the seminiferous tubules are gently crushed and spermatozoa centrifuged out to be prepared for

¹Some advocate the addition of sperms almost immediately (Chen and Sathanathan, 1986); obviously the timing depends on the state of the harvested oocytes.

ICSI or SUZI techniques as described below. The fact that testicular sperm are capable of fertilization, given capacitation techniques *in vitro*, has suggested that the sperm dysfunction of some infertile men is acquired in the reproductive tract, possibly by exposure to reactive oxygen species. The successes achieved in this field also indicate that the limiting factors for successful pregnancy are not the quality of spermatozoa but rather oocyte quality and endometrial receptivity in the woman.

Fertilization

The capacitated spermatozoa are mixed with the oocytes and incubated in 5% carbon dioxide in air at 37°C. Fertilization of the oocytes and cleavage of the resulting zygotes are monitored microscopically.

IVF is not necessary in some cases if infertility is due to impaired spermatozoon motility; oocytes harvested as described can be mixed with precapacitated spermatozoa and the mixture introduced directly into the ampulla of the oviduct where fertilization occurs. This is known as **gamete intrafallopian transfer (GIFT)**. The alternative is **zygote intrafallopian transfer (ZIFT)**, in which the oocytes are fertilized *in vitro* and only fertilized pronuclear zygotes are introduced into the ampulla.

In some cases of infertility the sperm are incapable of penetrating the zona pellucida. In these cases, harvested oocytes are allowed to mature to the second meiotic metaphase and then held in place in a culture dish with a minute suction pipette. A sharp glass needle is then used to cut a hole in the zona pellucida (a technique called **zona drilling**). A spermatozoon can then reach the oocyte through this hole, though care must be taken to avoid polyspermy. However, the fertilization rate in humans following zona drilling is only 15-30%, even if only the sperm that pass through the hole are counted. This supports the theory that the cumulus cells and zona pellucida act like a filter which only passes spermatozoa capable of fertilization.

Other techniques of fertilization are also used. In **sub-zonal insemination (SUZI)**, up to 10 spermatozoa that have been incubated in 0.1% pentoxifylline (to induce motility) and 50% follicular fluid are injected into the perivitelline space. In **intra-cytoplasmic sperm injection (ICSI)**, sperm incubated in 5mM CaCl₂ are placed in a micropipette and one spermatozoon is injected into the cytoplasm of the oocyte. Both techniques are used when the quality and motility of spermatozoa are low: ICSI is particularly effective even with non-motile sperm or spermatids.

Implantation

The zygotes are allowed to develop to the 2- to 8-cell stage and are then inserted into the uterus via the cervical canal. The probability of a successful pregnancy is enhanced by implanting two or three zygotes; the incidence of multiple pregnancy but also of spontaneous abortion is higher than that following normal ovulation and fertilization where the morula passes into the uterus via the uterine tube.

Preimplantation zygotes and blastocysts resulting from IVF may be preserved for long periods by freezing with a cryoprotectant solution such as glycerol or 1,2-propanediol (**cryopreservation**). It is now common practice to transfer thawed zygotes and blastocysts to the uterus successfully.

Conclusion

IVF is a relatively recent development in medical technology, and in Western society it is becoming increasingly important as the techniques improve, making more and more instances of infertility susceptible to treatment. Ethically, there is much debate as to how it should be used (the use of foetal oocytes, for example) but technically it has advanced rapidly and continues to do so.

[~1,100 words; written Nov '94.]

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