Spring 2022 – Systems Biology of Reproduction
Lecture Outline – Assisted Reproduction/Contraception
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
April 26, 2022
Week 16

Assisted Reproduction/Contraception

Assisted Reproduction –

- Infertility
- ART – Artificial Insemination and Other Technology
- IVF and ICSI
- Pre-implantation Diagnosis (PGD) and Cloning
- Stem Cells and Reproduction

Contraception

- Population Growth and Projections
- Need and History of Contraception
- Sterilization and Other Procedure Use
- Female Hormonal Contraception and Morning After Pill
- Female Implants
- Male Contraception and Targets
- Male Hormonal Contraception and Efficiency
- Other Male Contraceptive Targets

Required Reading

Pennings G, Segers S, Mertes H. (2018) Modern Ethical Dilemmas in ART. in: Encyclopedia of Reproduction 2nd Edition, Ed: MK Skinner. Elsevier. Vol. 5:391-394.

Duffy DM and Archer DF (2018) Female Contraception. in: Encyclopedia of Reproduction 2nd Edition, Ed: MK Skinner. Elsevier. Vol. 2:255-260.

Liu PY (2018) Male contraception. in: Encyclopedia of Reproduction 2nd Edition, Ed: MK Skinner. Elsevier. Vol. 1:478-485.

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Modern Ethical Dilemmas in ART

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Introduction

Medically assisted reproduction offers people an increasingly broad spectrum of techniques to have the child they long for. These techniques can be subdivided in two categories: those using the intended parents own genetic material/gametes and those using donor genetic material/gametes. For the large majority of the people, the applications in the latter category are "solutions of the last resort": only if there is no way that a couple or person can have a genetically related child will a non-genetically related child be considered. However, almost all the techniques to bypass a defect of the reproductive system imply manipulations and modifications of which the consequences for the long term health of the future offspring are unknown. One recent example that caused quite some concern is mitochondrial transfer. Women at risk of transmitting a mitochondrial disorder to their offspring either transfer healthy mitochondria from a donor into their oocytes or transfer the nucleus of their egg into an enucleated donor oocyte. The health consequences of this manipulation are unknown. The important point is, however, that this issue could be avoided by using donor eggs. Still, despite the risks, one goes ahead with the development of the technique. The same applies to people with known increased risks of transmitting a genetic disease who go through multiple cycles of IVF and PGD while donor gametes would solve the issue. At the same time, the use of donor gametes is not self-evident or easy either. In the present climate in which much emphasis is put on the person of the donor, the importance of knowing one's genetic origin in order to develop one's identity and the strong value attached to the genetic relationship between the donor and the child, gamete donation is increasingly confronted with moral and psychological problems. Moreover, there is a shortage of donor gametes (in particular of eggs) in many countries, resulting in long waiting lists.

In this article we will look more closely at two applications that illustrate this general problem: oocyte cryopreservation for healthy women and stem cell derived gametes. Both examples show in different ways the importance of genetic parenthood.

Oocyte Cryopreservation for Healthy Women

One of the interventions in ART that has been the topic of ethical debate for several years now is oocyte cryopreservation in anticipation of age-related fertility decline, known as "social egg freezing", "elective egg freezing", "non-medical egg freezing" or "AGE banking" (whereby AGE is an acronym for Anticipated Gamete Exhaustion). Although the first healthy live birth from a frozen human egg cell dates back to 1986, egg freezing has long been so inefficient that it was hardly considered a valid treatment option. From 2004 onwards, however, there has been an explosion in research directed at oocyte cryopreservation (OC), leading to successful slow freezing and vitrification protocols for human egg cells with high survival rates after thawing and with reassuring preliminary data on the health of the resulting offspring (Cobo et al., 2014). There are several applications for this new technology: donor egg banking, but also autologous egg banking. The latter was introduced in the context of oncofertility, so that women fearing premature infertility due to disease, gonadotoxic medical treatments or irradiation therapies could bank their eggs before irreversibly losing them.

Soon there was also an interest in storing eggs for women not faced with a medical condition leading to premature infertility, but for those approaching the natural boundaries of their reproductive lifespan while being unable to start a parental project, for example due to lack of a partner. However, whereas the introduction of OC for oncofertility was met with much enthusiasm and optimism, the expansion of the option of OC to healthy women did not incite the same reactions (Mertes, 2013). Both the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) initially denounced the use of OC for healthy women, while endorsing it for medical conditions. In 2012, ESHRE changed its recommendations, saying that OC should also be available for non-medical reasons, whereas the ASRM remains cautious (ESHRE Task Force on Ethics and Law et al., 2012; Practice Committee of the American Society for Reproductive Medicine and Practice Committee of the Society for Reproductive Technology, 2012).

Medical versus Social freezing: A Tenable Distinction?

A first important question is on which grounds a moral distinction can be made between "medical egg freezing" and "non-medical egg freezing". If women are only allowed to preserve their fertility when they anticipate premature infertility, but not when they anticipate natural infertility, one needs to give a justification for this differential treatment. Several arguments are possible, but not convincing. A first one is that we should respect natural boundaries. But why? Is medicine not constantly interfering with the natural decline of our bodies? A second one is that only those who are unable to anticipate (and thus prevent) being infertile before embarking on parenthood are deserving of a medical intervention. But why? First, medicine is constantly curing preventable medical problems. Second, the "solution" of reproducing early in life is not an option for everyone: there are very good reasons for waiting to establish a family until certain side constraints are in place, such as having a partner, financial stability, et cetera.

Moreover, there is a large gray area between medical and non-medical reasons for egg banking. In which category do women who freeze their eggs due to a family history of early menopause (and a low ovarian reserve) belong? What about women who have undergone a cancer treatment in the past that has diminished, but not depleted their ovarian reserve and who want to store eggs after their treatment because they fear premature ovarian failure? In short, it is difficult to indicate what the crucial difference between the two applications (medical and non-medical) is and even more so why it would matter.

Reasons for Caution

Yet there are reasons to be cautious about the introduction of AGE banking, with the main fear being that this new technology is being "oversold". Cobo et al. (2016) report cumulative live birth rates in women 35 or younger of respectively 15.4%, 40.8%, and 85.2% when freezing 5, 8 or 15 oocytes. The cumulative live birth rates achieved with 5, 8 and 11 oocytes for women 36 or older were 5.1%, 19.9% and 35.6% (in this group the additional benefit of banking more than 11 oocytes is very small). At present most women opting for AGE banking belong to the latter group, not the former, as reported average ages at the time of egg banking range from 36 to 38 years. Although these are good success rates compared to a subfertile population, this still means that most of these women have at best a 35% chance of achieving a healthy live birth (if their ovarian response is good enough to generate 11 oocytes). This contrasts with the image of egg freezing that commercial companies offering this technology create. Egg banking is said to "stop the biological clock" or offer "insurance" against infertility. The fear is that women will expect more of egg banking than the intervention can deliver and that many women will feel disappointed and betrayed when it turns out that the eggs they stored do not lead to a live birth. In this sense, an intervention that is being marketed as increasing women's reproductive autonomy becomes an intervention that curtails reproductive autonomy and preys on desperate women.

Should this technology be marketed to young women then, with higher success rates? In fact, this may equally lead to an undermining of a woman's intended reproductive life plan. When egg banking becomes a deliberate attempt to put parenthood "on hold" while other life goals are being pursued (possibly under pressure of employers), women may insufficiently be aware that egg banking in such a context jeopardizes a woman's reproductive potential, rather than safeguarding it, as a woman's chances of reproducing naturally at a younger age will always be better than her chances of reproducing with banked eggs at an older age. This fear became a lot less hypothetical when Facebook and Apple announced in 2014 that they would offer egg freezing to their female employees (Mertes, 2015).

What Does a Cautious Implementation Look Like?

The crucial question then becomes: how can this technology be implemented in a way that has maximal respect for reproductive autonomy and an acceptable utility rate? Regarding the utility rate the paradox is that the younger women freeze their eggs, the better quality the eggs will be, but the less chance that the woman will actually need them, as they have plenty of time to reproduce naturally. The older a woman is when she freezes her eggs, the worse their quality, but also the less chance she has of reproducing naturally (and the more chance to need her stored eggs). The ideal age for egg banking would therefore be between 30 and 35 (Mertes and Pennings, 2011). Also, women opting for egg banking should be counseled carefully about the drawbacks of AGE banking: about the success rates, tailored to their age/ovarian reserve; about the technicalities of the procedure, for example the fact that ICSI will be needed; about the maximal storage period, etc. Next to this practical counseling, it would also be beneficial to inspire candidates for AGE banking to critically reflect on the importance of (genetic) parenthood and on the prospect of the alternatives. This may imply that women approaching their forties or young women with many fertile years ahead of them will end up being discouraged from storing their eggs, while women who are most likely to benefit will be encouraged to bank. Given the possible biasing effect of commercial interests, it becomes increasingly important to ensure that counseling and information provision to women is as objective and complete as possible.

Stem Cell Derived Gametes

The in vitro creation of stem cell-derived (SCD) gametes promises new possibilities for human procreation, both via basic science applications and clinical use in assisted reproduction. The value of genetic relatedness is an important incentive in the development of this technique. Proof of principle for in vitro gametogenesis (IVG) is available in mice, and it is believed that the differentiation of functional human gametes from pluripotent stem cells will be possible in the "not too distant future" (Cohen et al., 2017). Creation of gametes from embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) is the main focus. Other sources such as bone marrow stem cells and germline stem cells receive less (ethical) attention. We will first outline the possible reproductive applications of IVG and then the ethical challenges of both ESC-derived and iPSC-derived gametes.

Patient-Specific SCD-Gametes

The most important goal of IVG is to establish a genetic link between the parents-to-be and the future offspring. To establish a genetic link, patient-specific SCD-gametes are needed. This requires either the creation of a cloned embryo through somatic cell nuclear transfer (SCNT) from which ESCs are derived or the creation of induced pluripotent stem cells (iPSCs) from a somatic

cell of the patient, which will then be differentiated into gametes. It is also suggested that IVG would make equal genetic relatedness between the child and both partners possible for same-sex couples, but researchers remain skeptical about reprogramming stem cells to gametes of the opposite sex (Moreno et al., 2015). Moreover, if IVG is to lead to the creation of patient-specific SCD-gametes at all, the technique of SCNT or the technique to obtain iPSCs would have to be improved.

If it would become possible to create patient-specific SCD-gametes, it could also be combined with technologies to prevent certain diseases in the future offspring. IVG could enable the creation of a large gamete and embryo pool to select (through PGD) genetic traits. Alternatively, stem cells could be modified via genome editing, and then differentiated into gametes (Vassena et al., 2016). This can be seen as an additional benefit, although it also raises concerns about possible usage to select/edit non-disease related traits.

Non-Patient-Specific SCD-Gametes

Deriving gametes from ESCs of embryos created by fertilization is presumably more feasible and closer to clinical application than creating patient-specific SCD-gametes. This would require the use of donated spare IVF embryos, or embryos created from oocytes and sperm donated for this goal. However, these gametes would be non-patient-specific, and will thus not lead to shared genetic parenthood.

Yet, the value of parent–child genetic relatedness also plays a central role here. Non-patient-specific SCD-gametes could be used to create an SCD-gamete bank for "third party" assisted reproduction. One benefit of this scenario over "traditional" donor assisted reproduction is that the parents-to-be would not have to fear that the donor would claim parental rights based on his/her genetic link with the child, since the donor (the embryo) is destroyed in the process of gamete derivation (unless such rights would be accorded to the genetic grandparents, viz. the embryo donors). Also, the resulting child would have no "unknown" genetic progenitor to look for, which might be more comforting than knowing that one's genetic parent is "out there". It could, however, be countered that this benefit is undermined by the child's inability to enrich his/her narrative by knowing that one of his/her genetic parents never existed as a person.

Non-patient-specific SCD-gametes could also be used for same-sex reproduction: a gamete derived from a fertilized embryo, created by combining a donor gamete with one of the partner's gametes, could be combined with the other partner's complementary gamete (Segers et al., 2017b). This would lead to a 50% genetic link between the latter partner and the child, while both the other partner and the donor would share 25% of the child's DNA. Many would consider it an important advantage of this scenario that although a donor is still involved, it will not be possible to ascribe a greater parental status to him/her solely on the basis of his/her genetic contribution. The partner who contributed as much to the child's genetic makeup as the donor, would outrank the donor given his/her role as a social parent and the intention to be a parent.

New Possibilities of Reproduction

Application of IVG for reproductive purposes could revolutionize human procreation. Apart from possible use in same-sex reproduction and treatments for infertile couples of reproductive age, IVG could allow anyone to produce gametes, regardless of age or relationship status (Smajdor and Cutas, 2015). If person-specific gametes could be created from stem cells, this could allow women to reproduce later in life, despite declining oocyte quality and even if they have already gone through menopause (see above). Prepubescent children would not be restricted by their age to have genetically related offspring either. IVG could also allow variations in the number of people involved in genetic parenting. Fusing someone's natural gamete with a derived gamete from this same person could result in single genetic parenthood. Also multiple genetic parenting might become possible: gametes of multiple persons could be combined to create embryos, from which ESC-derived gametes could be established, which could again be recombined in as much cycles as needed to obtain a child who is genetically related to each of the participating persons. For a more extensive ethical discussion of these scenarios, see: Smajdor and Cutas (2015) and Segers et al. (2017a). In the final section, we will explore some of the general ethical concerns about IVG.

Main Ethical Concerns

For reproductive IVG, the main ethical issue is the safety risk to the offspring. At this stage, clinical application of IVG is unacceptable because of the potential high risk to the offspring's physical wellbeing. This is especially the case for patient-specific SCD-gametes, as both the technique of SCNT and that of iPSCs derivation are hindered by incomplete reprogramming of the somatic cell nucleus to an embryonic state. The derivation of gametes from ESC-lines from embryos created by fertilization would be less complicated and therefore possibly safer. Still, this raises the question what standard of risk is acceptable to make genetic relatedness possible, or, in the case of non-patient-specific reproductive IVG, to avoid the attribution of parental status to the donor. It could be argued that, unless their life is not worth living, IVG offspring cannot be harmed by the parental choice to reproduce through IVG because without this decision they would not have existed at all. However, this conclusion runs counter to most people's moral intuition. Instead the "reasonable welfare principle" has been suggested: this standard is adopted by the European Society of Human Reproduction and Embryology (ESHRE) and holds that the use of a reproductive technology is only acceptable if the resulting child will have a reasonably happy life (Pennings et al., 2007).

Even if IVG would be safe, it is worth contemplating that the endeavor of creating SCD-gametes for reproduction might reinforce the dogma of genetic relatedness. Even the use of non-patient-specific SCD-gametes could, at least implicitly, propagate the importance of genetic ties in a parent–child relationship. This is not morally wrong per se, and is also not unique to IVG, but it could send the incorrect message that a good parent–child relationship requires a genetic link. According to this reinforcement argument, IVG would create rather than meet demand, which is a fortiori important given the related questions of access, cost and resource allocation. Because IVG will probably not be affordable for everyone who could benefit from it, it could be argued that access to IVG should be state covered. It is questionable, however, whether the goals that are served by IVG are important enough to outweigh other claims on public resources. When this weighing is done, not only the goal of reproduction should be considered, but also the possible benefits of IVG in the research context.

A final argument is that IVG as such is unacceptable because it inherently involves embryo destruction. Embryo destruction is obvious if ESC-derived gametes are used (both patient-specific and non-patient-specific), but also the iPSC route might be troubling for those who oppose embryo destruction. While iPSC-derived gametes generally avoid the use of embryos, embryos will still have to be created in the research phase to ensure the safety of the technique. Many people find it less disrespectful to use spare IVF embryos (as they will be destroyed anyway), but those applications involving the destruction of embryos created from SCD-gametes that were differentiated from spare embryos will likely be opposed by those who object to the intentional creation/destruction of embryos. Only if the prohibition of embryo destruction is not regarded as an absolute rule, and if the importance of the goals of IVG (whether reproductive or scientific) outweigh the cons of creating and destroying embryos, can those who oppose embryo destruction accept IVG in general.

Conclusion

Having a genetically related child is a highly valued goal in many people's lives. Still, it is not easy to justify as the benefits of this wish are unclear. When the wish is accompanied by possible health risks for the offspring, society and the future parents will have to decide what risk is acceptable. In this balancing act, the existence of alternatives has to be taken into account. Donor gametes are such an alternative in most circumstances. However, this solution is more complicated from the psychosocial point of view given the increasing value attributed to genetic relationships.

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Female Contraception

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Glossary

Actual efficacy (also called practical efficacy or typical use efficacy) Contraceptive efficacy when a method is used by people not involved in clinical trials.

Breakthrough bleeding Unanticipated or unscheduled bleeding or spotting, originating in the uterus and similar to menstrual bleeding.

Efficacy Effectiveness of a contraceptive method, the expected number of women who do not get pregnant in a group of 100 women using the method for an entire year.

Estrogens A group of structurally related steroid hormones that act at estrogen receptors.

Endometrium Lining of the uterus, is lost with menstruation.

Intrauterine device (IUD) T-shaped device placed in the uterus for contraception.

Long-acting reversible contraception (LARC) Contraceptives that remain in place and provide long-lasting but reversible contraceptive action.

Luteinizing hormone (LH) A protein hormone produced by the anterior pituitary.

Progestins A group of structurally related steroid hormones that act at progesterone receptors.

Subcutaneous Under the skin.

Synthetic Human-made in the laboratory, not found in nature.

Theoretical efficacy Contraceptive efficacy during perfect use, usually determined in clinical trials.

Transdermal Across the skin.

Opportunities for Female Contraception

Fertility requires interaction between the oocyte and the sperm. During intercourse, ejaculation results in the release of hundreds of millions of sperm in the vagina, near the opening of the uterine cervix. Sperm penetrate the cervical mucous and move through the lumen of the uterus into the oviduct (also called the fallopian tube or uterine tube). The oocyte (also known as the egg or ovum) leaves the ovary at ovulation, enters the oviduct through the fimbria, and is propelled through the oviduct and toward the uterus by the movement of cilia and muscle contractions of the oviduct. Fertilization, the fusion of oocyte and sperm, occurs in the oviduct. After fertilization, the single cell embryo, or zygote, continues to move through the oviduct and toward the uterus as the early cell divisions of embryonic development get underway.

The oocyte develops within the ovarian follicle. The mature ovarian follicle (also called a dominant, ovulatory, or Graafian follicle) produces large amounts of the steroid hormone estrogen, which enters the circulation. High serum levels of estrogen stimulate the anterior pituitary to release of a large amount or "surge" of LH. This surge of LH acts at the ovarian follicle to initiate a series of events that culminate in ovulation, including rupture of the follicle and release of the oocyte. After ovulation, the ruptured follicle is transformed into a corpus luteum and produces large amounts of the steroid hormone progesterone.

Estrogen and progesterone stimulate changes in the woman's body that affect fertility. Estrogen stimulates the oviduct to produce fluid and increases muscle contractions, which assist the movement of the oocyte from the fimbria and toward the uterus. Estrogen results in the production of a thin, watery cervical mucous, which is easily penetrated by sperm moving from the vagina into the uterus and toward the oviduct. Estrogen causes the endometrial lining of the uterus to regrow after menstruation. In turn, progesterone reduces fluid secretion and muscle contractions in the oviduct, limiting oocyte entry into the oviduct and transport through the oviduct. Progesterone increases the viscosity of cervical mucous, reducing sperm transit from the vagina to the uterus and oviduct. Progesterone also acts at the endometrial lining of the uterus to prepare for implantation and pregnancy. Falling progesterone levels after a period of progesterone exposure causes shedding of the uterine endometrium or menstruation. However, continuous exposure to progesterone prevents growth and development of the uterine endometrium.

Structure and function of the female reproductive tract offers many opportunities to intervene and prevent fertility (Table 1). Barrier methods prevent movement of sperm from the vagina, thereby preventing fertilization. Occlusion of the oviducts also prevents sperm–oocyte interaction. Spermicides damage or destroy sperm cells, so sperm are unable to fertilize the oocyte. Chemical spermicides and the intrauterine device (IUD) utilize this approach to fertility prevention. Hormonal contraception disrupts many aspects of female reproductive function, ultimately reducing fertility. Behavioral methods to prevent pregnancy include abstinence, periodic abstinence, and withdrawal of the penis from the vagina prior to ejaculation.

Table 1 Most effective contraceptive method used in the past month by US women in 2012

Method	*Number of users*	*Percent of women aged 15–44*
The pill	9,720,000	16.0
Female permanent contraception	9,443,000	15.5
IUD	3,884,000	6.4
Withdrawal	1,817,000	3.0
Progestin injection	1697,00	2.8
Vaginal ring	759,000	1.2
Periodic abstinence	509,000	0.8
Progestin implant	492,000	0.8
Hormone patch	217,000	0.4
Emergency contraception	91,000	0.2
Other female methods[a]	133,000	0.3
Male methods[b]	8,823,000	14.5
No method; at risk of unintended pregnancy	4,175,000	6.9
No method; not at risk of unintended pregnancy	19,126,000	31.4

[a]Includes diaphragm, female condom, spermicide, cervical cap, and sponge.

[b]Includes male condom and male sterilization (vasectomy).

Table adapted with permission from Guttmacher Institute, Contraceptive use in the United States, Fact Sheet, New York: Guttmacher Institute, 2016, https://www.guttmacher.org/fact-sheet/contraceptive-use-united-states.

Selection of a Contraceptive Method

There are many considerations for selection of a contraceptive method. Permanent contraception eliminates the possibility of all future pregnancies. Reversible contraceptives can prevent pregnancy in the short-term while preserving long-term fertility. Contraception can be used with the intention of preventing all pregnancies. However, reduction of overall family size by increasing time interval between pregnancies can also be achieved with contraception. When selecting a contraceptive method, a woman may also consider safety, effectiveness, cost of the method, how often the user needs to employ the contraceptive, whether the method requires action immediately before intercourse, side effects caused by use of the method, impact on the sexual experience, if her partner should be involved, and whether a clinician must be consulted to acquire or employ the method properly.

Steroid Hormones as Components of Contraceptives

Steroid hormones, including estrogen and progesterone, are important to the proper function of the female reproductive system. High levels of estrogen are characteristic of the first half of the menstrual cycle. During this period of follicle growth and maturation, estrogen supports the health of reproductive tract organs including the oviduct, uterus, and cervix. Estrogen also increases the number of progesterone receptors in these organs. After ovulation, the follicle transforms into the corpus luteum, which produces large amounts of progesterone. Reproductive tract organs respond to progesterone via progesterone receptors to decrease oviductal motility, thicken cervical mucous, and prepare the uterine endometrium for possible implantation. Progesterone also decreases the number of estrogen receptors in these tissues, decreasing the effects of estrogen and limiting the ability of estrogen to increase progesterone receptor numbers.

Progesterone or a progesterone-like molecule (progestins) is an essential component of all hormonal contraceptives. As described earlier, progesterone decreases many reproductive tract functions required for fertilization. Progesterone also decreases estrogen action at these tissues due to the decrease in estrogen receptors. Finally, progesterone prevents the release of surge levels of LH, so LH cannot initiate events within the ovary that lead to ovulation, including follicle rupture and release of the oocyte. Many hormonal contraceptives also include an estrogen to reduce unwanted and unpredictable uterine bleeding (often called breakthrough bleeding) and to help maintain the overall health of nonreproductive tissues, such as the bone, skin, and heart.

The development of synthetic, orally active progestins and estrogens was a major advance in female contraception. Progestins, including progesterone and 17α-hydroxyprogesterone, and estrogens, including estradiol and estrone, are produced in the body primarily by the ovary. For a contraceptive, circulating levels of steroid hormones must be maintained at relatively constant levels. Natural progestins and estrogens circulate in the bloodstream for several hours after production and then are removed from circulation by the liver and kidneys. Natural steroid hormones are damaged by passage through the digestive system, so oral administration of progesterone and estrogen is impractical. For these reasons, use of naturally occurring steroid hormones would require frequent readministration.

These limitations were overcome with the development of synthetic progestin and estrogen molecules. Synthetic hormones maintain bioactivity after oral administration, passage through the digestive system, and uptake into the circulation. Synthetic hormones can also be administered via transdermal patches, subcutaneous implants, and injections. Synthetic hormones remain

in the circulation longer than natural steroids because their chemical structure prevents rapid modification, conjugation, and elimination in the liver and kidneys. Synthetic progestins and estrogens activate progesterone receptors and estrogen receptors, respectively, but often with higher potency than natural progesterone and estrogen. These are just some of the reasons that synthetic progestin and estrogen molecules are used as components of hormonal contraceptives.

Synthetic progestins that bind to progesterone receptors but block activity of progesterone receptors are called antiprogestins. These synthetic hormones can prevent fertility by disrupting the activity of progesterone in reproductive tissues. The most common contraceptive actions of antiprogestins are to (1) reduce or eliminate the ovulatory LH surge and (2) block necessary actions of progesterone within the ovulatory follicle prior to ovulation. Actions of antiprogestins in the uterus to disrupt implantation or early stages of placenta development are not contraceptive since these actions occur after fertilization has taken place.

Hormonal Contraception: Combination Hormonal Contraceptives

The first combination oral hormonal contraceptive, commonly known as The Pill, contained large amounts of a synthetic estrogen and a synthetic progestin. Since The Pill was introduced in 1960, there has been a steady decrease in the concentration of the progestin and the estrogen in oral contraceptive products. Products with lower steroid doses remain highly effective at preventing pregnancy with a substantial reduction in side effects, especially deep vein thrombosis and other cardiovascular events.

The regimen of oral hormonal contraceptives was initially designed to mimic the 28-day interval between menstruations. A common formulation involved 21 days of steroid-containing pills, with 7 days of placebos to allow circulating steroid hormone levels to fall and permit menstruation. Changes in duration of administration have resulted in a variety of regimens, including 24 days of steroids with 4 days of placebo, continuous steroid administration for 84 days, and continuous steroid administration for 365 days. Continuous pill use increases the incidence of no menstrual bleeding, which is highly acceptable to some women. However, unscheduled endometrial bleeding, known as breakthrough bleeding, can occur with any steroid regimen. Oral hormonal contraceptives containing estrogen and progestin have a pregnancy rate of 1%–4% in well-controlled clinical trials (Table 2).

Hormonal contraception is also available in nonpill forms. Estrogen plus progestin contraceptives can be administered via a transdermal delivery system, vaginal ring, or injection. Transdermal and vaginal delivery use a regimen of 21 days of active hormone and 7 days of no hormone to permit menstruation and approximate the length of the typical menstrual cycle. The monthly injection has a duration of efficacy of 28 days and must be administered every 4 weeks. The contraceptive efficacy of these methods is very high, with pregnancy rates of 1%–2% in well-controlled clinical trials.

The lower dose combination oral hormonal contraceptive products in current use are relatively safe. The principal significant adverse events resulting from use of combination oral hormonal contraception are increased incidence of venous thrombosis in the leg and pulmonary emboli. Use of a modern, low-dose combination oral hormonal contraceptive doubles a woman's risk for these cardiovascular events. However, the risk of cardiovascular events associated with these contraceptives is much lower than the risk of cardiovascular events associated with pregnancy. Women with certain inherited clotting disorders or a family history of certain cardiovascular disorders should consider other methods of contraception. Women over the age of 35 who have hypertension, have diabetes mellitus, or smoke cigarettes are at increased risk of stroke and myocardial infarction. In women without these risk factors, low-dose combination oral hormonal contraceptives can be used during a woman's reproductive years up to menopause without a significant age-related increased risk of cardiovascular disease.

Table 2 Pregnancy rates (number out of 100 women) during the first year of continuous use of the contraceptive method

Method	*Perfect use*[a]	*Typical use*
Intrauterine device—levonorgestrel	0.1	0.1
Progestin implant	0.1	0.1
Progestin injection	0.3	0.3
Female permanent contraception[b]	0.5	0.5
Intrauterine device—copper	0.6	0.8
Oral combined hormonal	1	9
Oral progestin only	0.5	9
Withdrawal	4	19
Diaphragm with spermicide	6	20
Female condom	5	24
Periodic abstinence	5	25
Spermicide alone	6	26
No contraception	85	85

[a]From product label or other information.
[b]Includes both surgical and nonsurgical methods.

Side effects include nausea, weight gain, mood disturbances, breast tenderness, headache, and unscheduled endometrial bleeding. These side effects may disappear or improve with continued use. However, for some women, side effects are significant and result in discontinuation of the method.

Typical failure rates for oral, transdermal, vaginal, or injectable combined hormonal contraceptives are approximately 9% and are likely the result of compliance errors by the user.

Hormonal Contraception: Progestin-Only Methods

Progestins inhibit the pituitary LH surge and, therefore, prevent ovulation as the principal mechanism of action. Additional actions of progestins to decrease the likelihood of fertilization contribute to the high effectiveness of progestin contraceptives. Clinical testing of synthetic progestin-only oral methods reports pregnancy rates of $<1\%$. Typical user's failure rates for daily oral progestin-only methods are about 9%.

Contraceptive efficacy can be enhanced with delivery systems that provide extended duration of progestin activity and minimize or eliminate the need to remember to use the contraceptive. Progestins can be delivered by subcutaneous implants, vaginal ring, IUD, and injection. These methods are termed long-acting reversible contraceptives (LARCs). Subcutaneous implants, vaginal rings, and IUDs contain the progestin within a plastic membrane. The plastic membrane controls the amount of hormone released each day, resulting in a duration of action of 1 month to 8 years, depending on the individual device. The high efficacy associated with these methods is due, in large part, to the fact that the user does not need to remember to use the method on a daily or weekly basis. Implants, vaginal rings, and IUDs can be removed for relatively rapid return to fertility. However, injections form a subcutaneous depot of progestin that cannot be removed. As such, return to fertility after discontinuing injections can require up to a year. The delivery system has a significant effect on typical user pregnancy rates with progestin-only contraceptives, making LARCs highly effective. Theoretical and typical user pregnancy rates are both $<1\%$.

The primary side effect of progestin-only contraceptives is unscheduled and irregular endometrial bleeding. This bleeding does not reflect a malignancy but is the principal reason for discontinuation of progestin-only methods. Progestins have none of the estrogen-related significant side effects, most importantly blood clots. For this reason, hormonal contraceptives that contain progestin with no estrogen may be appropriate for women at higher risk of cardiovascular issues, including older women and women who smoke. Progestin-only contraceptives are appropriate for breastfeeding women since the estrogen component of combined hormonal contraceptives interferes with lactation.

Intrauterine Devices

The IUD is a T-shaped plastic device that is placed within the lumen of the uterus, such that the arms of the "T" point toward the oviducts, with the lower end of the "T" pointing toward the cervix. The plastic of the IUD has contraceptive action, creating a sterile inflammatory response that has spermicidal activity. However, all currently available IUDs are designed to have enhanced activities. Some IUDs include copper wound on the arms and stem, which enhances the spermicidal activity. Other IUDs include a progestin within the stem of the device. With progestin-releasing IUDs, locally elevated progestin within the uterine lumen decreases endometrial development and reduces or eliminates menstruation; progestin also decreases uterine contractions to reduce the rate of spontaneous expulsion of the IUD.

An IUD should be placed in the uterus by an experienced clinician. For insertion, the arms of the T are folded parallel to the stem of the device and held in place with an inserter. The inserter is introduced through the cervix and into the uterine cavity. Once properly placed, the IUD is deployed and achieves its active T shape. Each IUD has a different length of time approved for use, with a range of 5–10 years. Fertility is typically restored within a few weeks of IUD removal. IUDs provide highly effective protection against pregnancy, with theoretical and typical user pregnancy rates $<1\%$.

Barrier Methods

Perhaps the best known barrier method is the male condom. The female condom is also a barrier contraceptive method. Female condoms are made out of latex, nitrile, or polyurethane. The female condom is designed to fit into the vagina, with a plastic ring anchoring it in the upper vagina. The condom covers the vaginal walls and extends outside of the vagina where a second ring prevents it from being pressed inward at the time of intercourse. Similar to the male condom, sperm are retained within the female condom after ejaculation, preventing sperm transit from the vagina to the cervix. Clinical studies of consistent use are associated with pregnancy rates of 4%–5%, while typical use pregnancy rates of 21% for the female condom have been reported. Condoms, including both male and female condoms, are highly recommended for use by couples at risk for sexually transmitted infections.

The diaphragm is a round device with a pouch, and the outer edge contains a spring designed to hold the device in place in the upper vagina. The uterine cervix fits into the diaphragm pouch, which is filled with a spermicide by the user prior to insertion. The diaphragm should be inserted before penile penetration and left in place for 1 h after ejaculation. Diaphragms are reusable and come in a variety of sizes. A diaphragm should be fitted by a clinician; the fit should be checked regularly, especially after pregnancy

or significant weight gain/loss. Diaphragms used consistently and correctly have a failure rate of 6%. Typical use failure rates are 20% for the diaphragm.

The cervical cap is a reusable contraceptive device similar to the diaphragm, except that the cap is smaller and fits tightly around the opening to the cervix. Effectiveness is enhanced when used with a spermicide. A cervical cap should be fitted by a clinician and refitted after pregnancy. Failure rates with consistent and typical use are slightly higher than for the diaphragm.

Side effects of barrier methods are minimal. Individuals with allergies to latex should select products that do not contain latex.

Spermicides

Spermicides are detergents that damage the cell membrane of the sperm. Spermicides must be placed within the vagina before penile penetration to be effective. The spermicide most commonly available is nonoxynol-9.

Spermicidal products include foam, creams, gel, vaginal suppositories, and vaginal film. When a spermicide is used as the only method of contraception, the pregnancy rate is 6% in clinical studies but 26% with typical use. Spermicides are often combined with a barrier method, such as a condom or diaphragm.

Spermicides are also impregnated into other contraceptive products to enhance efficacy. The contraceptive sponge is a single-use contraceptive that is inserted into the vagina to cover the uterine cervix. The sponge contains spermicide, which disables or destroys sperm during transit from the vagina to the uterus. The sponge must be inserted prior to intercourse and should be left in place for at least 6 h after intercourse. In women who have never given birth, consistent and correct use of the contraceptive sponge has a failure rate of 1%–9%; typical use failure rates are 11%–24%. Typical failure rates are higher for women who have experienced pregnancy (24%–32%).

Side effects with spermicides are minimal.

Emergency Contraception

Emergency contraception is intended for use after unprotected intercourse, in cases where a contraceptive method was not used or when the primary contraceptive method may have failed.

Two forms of oral emergency contraception, also called morning-after pills, are currently available in the United States. A variety of products, including Plan B®, contain the progestin levonorgestrel as the active ingredient. Another option is ulipristal acetate, a progesterone receptor modulator, currently marketed as Ella®. These progestins prevent pregnancy by preventing the LH surge and, therefore, ovulation. For this reason, emergency contraceptive pills are most effective when taken within 72 h of intercourse. Emergency contraception does not interfere with fertilization or implantation, so these medications are less effective when taken more than 3 days after intercourse. Emergency contraceptives are also less effective in obese women. Levonorgestrel-containing emergency contraceptives reduce the estimated pregnancy rate by 33%. Ulipristal acetate is more effective, reducing the estimated pregnancy rate by 66%. Side effects are similar to those reported for other hormonal contraceptives and include nausea, headache, uterine cramping, unscheduled uterine bleeding, fatigue, and breast tenderness.

The copper IUD is a highly effective emergency contraceptive method. The copper IUD can be inserted up to 5 days following intercourse as an emergency method. Following insertion, the copper IUD will provide continued contraceptive action for up to 10 years unless removed.

Permanent Contraception

Permanent female contraception (also called sterilization) involves obstruction of the oviducts. Surgical tubal ligation is typically performed as a laparoscopic outpatient procedure. During surgery, the oviducts are severed or banded to prevent transit of oocytes or sperm, and major side effects are those of surgery. Nonsurgical methods involve introduction of a device inserter through the cervix, a procedure called a hysteroscopy. During the procedure, the contraceptive device is placed into the opening between each of the oviducts and the uterus. The contraceptive device creates a local inflammatory response and facilitates fibrosis, which blocks the oviduct. Complete blockage of both oviducts can take up to 3 months to be complete, so the woman should use an alternative, effective form of contraception during this period. Reversals of tubal obstruction have been reported. However, tubal obstruction by any method should be considered to be permanent. Female permanent contraception is highly effective, with a pregnancy rate of $< 1\%$.

Removal of the uterus (hysterectomy) for contraceptive purposes is not performed in the United States.

Behavioral Contraceptive Methods

The most utilized nonhormonal contraceptive method is withdrawal or removal of the penis from the vagina prior to ejaculation. While well-controlled clinical trials are lacking, this method is thought to have an overall contraceptive failure rate of approximately 19%.

Periodic abstinence, also known as natural family planning, the rhythm method, or the symptothermal method, is based on the concept that intercourse can result in pregnancy only during a few days in each menstrual cycle. Pregnancy can be avoided by (1) predicting when a woman is fertile and (2) abstaining from intercourse, using a barrier contraceptive or using withdrawal during the fertile period.

The fertile period of each menstrual cycle is defined as the days when intercourse can result in fertilization and formation of an embryo. Sperm travel from the vagina to the oviduct, where fertilization occurs. Sperm can survive in the oviduct for about 5 days, waiting for the oocyte to travel from the ovary after ovulation. The 5 days where intercourse can result in fertilization include the 4 days before ovulation and the day of ovulation.

Predicting the day of ovulation requires regular, consistent, and noninvasive monitoring of the woman's menstrual cycle. Purchased kits can be used to measure urinary LH and determine the day of the LH surge. Progesterone increases basal body temperature, so daily oral temperature measurements can be charted to determine the days of the luteal phase. Cervical mucous is watery during the follicular phase and viscous during the luteal phase. A sample of cervical mucous can be assessed for presence and consistency in order to determine if a woman is in the follicular phase or luteal phase of her menstrual cycle. Menstruation signals the first day of the follicular phase. Charting these changes on a calendar can document if a women has consistent, regular cycles of similar length. If so, then a prediction of the fertile period can be made for future menstrual cycles, with the fertile period beginning at least 3 days before the expected LH surge and lasting for a total of 5 days. During the fertile period, a woman can choose to avoid intercourse or use an alternative form of contraception to avoid pregnancy.

Few women have such predictable menstrual cycles, contributing to a high pregnancy rate of 25% for this method for contraception. However, periodic abstinence can be used successfully by highly motivated couples to increase time between pregnancies and reduce overall family size.

Summary

There are a variety of contraceptive options currently available for sexually active women. Choices for reversible contraception include highly effective hormonal methods and less effective barrier methods. IUDs and other LARCs provide effective, long-lasting, and reversible pregnancy prevention. Methods that obstruct the oviducts are highly effective and should be considered permanent. When selecting a method, effectiveness and reversibility should be balanced with other considerations, including safety, cost, and side effects.

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Male contraception

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Introduction

The Need for Contraception

Unintended pregnancies are unwanted, mistimed, or both unwanted and mistimed. Almost half of all pregnancies that occur in industrialized nations are unintended. Unintended pregnancies increase the risk for lower birth weights, poorer health outcomes, retarded development, and compromised education in the offspring, as well as postpartum depression and ongoing mental health problems in the mother. These adverse health and social consequences impact the entire family, and result in substantial societal costs that have been estimated to exceed $15 billion in a single year in the United States alone. Broadening contraceptive choice by allowing both men and women to fully share family planning responsibilities would promote important, but largely unmet individual, couple, and societal needs; reduce the number of elective terminations; and decrease overpopulation.

In the United States, 30% of couples rely solely on a male-directed method (male sterilization, condom, withdrawal). However, the proportion of all couples that are using male-directed methods varies greatly from 0% to 50% throughout the world. Given this variability, the median (rather than the average) proportion of couples using male-directed methods across over 60 nations surveyed is approximately 10% (United Nations, 2015). After excluding couples that are not using any male or female-directed contraceptive method, about 20%, and up to 80% in some countries, rely on a male-directed method. These data confirm that many couples already depend upon male-directed methods despite such methods being far from ideal. Usage would presumably be even greater if male-directed methods could be developed that were more effective, convenient, reversible, and safe.

Features of an Ideal Contraceptive

An ideal male-directed contraceptive is safe, rapidly and consistently effective (i.e., usable by all men) and rapidly and consistently reversible (i.e., reversible in all men). In contrast, the two currently available male-directed methods are not widely acceptable because vasectomies are not easily reversible and condoms are not very effective. During the last few decades, many new female-directed reversible hormonal contraceptive options including pills, patches, injections, implants, and intrauterine devices with various drug and dose combinations have been marketed. In contrast, there has not been a single reversible male-directed contraceptive developed over this same period. Currently, the only reversible male-directed method available is the condom, which was invented 400 years ago and has failure rates as high as 20% in the first year. New and effective options are needed to broaden choice for couples.

Male Hormonal Contraception

Summary

More than 2000 eugonadal men with unimpaired fertility at baseline have been exposed to over 2000 person-years of male hormonal contraception (MHC, consisting of androgens with or without progestins) to assess contraceptive efficacy (i.e., the ability to prevent pregnancies) (**Table 1**). These studies show the effectiveness, reversibility and short-term safety of MHC. MHC satisfies many of the requirements for an ideal contraceptive method that is male-directed. The hope is that a range of MHC treatment options will become available to fulfill differing needs and preferences of couples.

Androgen-progestin MHC treatment regimens reduce sperm output and induce a predictable degree of infertility by exploiting the negative feedback suppression of pituitary gonadotropin secretion by sex hormones. The goal of male hormonal contraceptive methods, according to expert consensus opinion, is to achieve and maintain severe oligozoospermia, defined as a sperm concentration in the ejaculate that is below 1 million/mL, in all users. If this can be consistently achieved and maintained, then contraceptive failure (i.e., pregnancy) rates of 0.6 (95% confidence interval CI 0.09–2.7) % per year can be expected (WHO Task Force on Methods for the Regulation of Male Fertility, 1996). These failure rates are comparable to those achieved with perfect use of female hormonal contraceptive methods. Even higher failure rates of 1.4 (0.4–3.7) % occur with a higher threshold of 3 million/mL. These data indicate that the degree of infertility induced can be predicted from the semen analysis, and is consistent with semen analysis being the most widely used clinical measure of spermatogenesis and male fecundity.

Mechanism of Action

Spermatogenesis is tightly regulated by the hypothalamo-pituitary-gonadal network through stimulatory feedforward and inhibitory feedback signaling (Liu and Veldhuis, 2014). The decapeptide, gonadotropin-releasing hormone (GnRH), is secreted in a pulsatile and synchronized fashion by approximately 1200 specialized neurons in the arcuate-nucleus of the mediobasal hypothalamus of the human. GnRH is secreted in minute quantities directly into a portal microvasculature system, where anatomical proximity

Table 1 Contraceptive failure rates of MHC

References	Regimen	Enrolled (n)	Target sperm concentration (M/mL)	Documented failure to suppress by 6 months n (%)	Median time to enter efficacy (months)	Maximum treatment duration (months)	Entered efficacy (n)	Sperm rebound during efficacy n (%)	Pregnancies during efficacy n (%)	Failure rate/100 couple years
Gu et al. (2009)	[a]TU	1045	1	43 (4)	3.6	30	855	10 (1.2)	9 (1.1)	1.1 (0.4–1.8)
WHO (1996)	[b]TE	399	3[c]	<8 (2)[c]	2.2	18	349	4 (1.1)	4 (1.1)	1.4 (0.4–3.7)
Behre et al. (2016)	[d]TU + NET-EN	320	1	9 (3)	3	18	266	6 (2.3)	4 (1.5)	2.2 (0.8–5.8)
Gu et al. (2003)	[a]TU	308	3	9 (3)	2–3	12	296	6 (2.0)	1 (0.3)	2.3 (0.5–4.2)
WHO (1990)	[b]TE	271	0	68 (25)	<6	18	157	11 (7.0)	1 (0.6)	0.8 (0.02–4.5)
Turner et al. (2003)	[e]Ti + DMPA	55	1	2 (4)	1.8	18	51	0[f]	0	0 (0.0–8.0)
McLachlan et al. (2000)	[g]Ti + various	36	1	8 (22)	Not reported	18	21	4 (19.0)	0	Not reported

[a]Testosterone undecanoate 500 mg/month (with 1000 mg loading dose).
[b]Testosterone enanthate 200 mg/week.
[c]Original threshold was 5 M/mL, but data shown here is for those who suppressed to no more than 3 M/mL.
[d]Testosterone undecanoate 1000 mg and norethisterone enanthate 200 mg every 8 weeks.
[e]Testosterone implant 800 mg every 4 or 6 months with depot medroxyprogesterone acetate 300 mg every 3 months.
[f]Four had sperm rebound and symptoms of androgen deficiency in the group receiving testosterone implants 800 mg every 6 months.
[g]Testosterone implant 800 mg or 1200 mg every 3 months with or without finasteride.

allows entrainment of pituitary gonadotropes to secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Pulsatile LH and pulsatile FSH circulate and act on distant testicular Leydig and Sertoli cells, to stimulate testosterone production and spermatogenesis, respectively. Circulating testosterone inhibits both hypothalamic GnRH, and pituitary LH and FSH secretion. The feedback inhibition of pituitary gonadotropins is through local aromatization to estradiol. Both FSH and high intratesticular levels of testosterone are required for spermatogenesis.

Male hormonal contraception exploits this negative feedback inhibition to impair spermatogenesis, in a manner that is analogous to female hormonal methods that prevent ovulation. The administration of exogenous androgens, with or without the addition of progestins, suppresses GnRH, LH, and FSH, reduces testicular testosterone concentrations and impairs spermatogenesis. In this context, exogenous progestins act to augment inhibition of the secretion of pituitary gonadotropins, so that unequivocally supraphysiological exogenous testosterone administration is no longer required. Other compounds could also be used alone or in combination to suppress pituitary gonadotropins more completely. Some of these, such as GnRH antagonists or non-pulsatile GnRH analogues that downregulate the GnRH receptor, have already been shown to reduce sperm output in humans, whereas other putative compounds that act upstream of the GnRH neuron through the kisspeptin-neurokinin B-dynorphin pathway remain to be directly tested for contraceptive efficacy. In all male hormonal contraceptive methods, testosterone or another androgen must be administered to maintain systemic testosterone exposure, in order to prevent the symptoms and consequences of unwanted hypogonadism.

Suppression of LH and FSH acutely decreases type B spermatogonia and blocks spermatogonial differentiation. A reduction in round and elongated spermatid production, and ultimately spermatozoa concentrations in the ejaculate, will therefore occur, but requires several weeks due to the duration of the spermatogenic cycle. Whereas azoospermia, the complete absence of spermatozoa in the ejaculate, would render fertilization impossible, this has not been consistently achievable with MHC in all users, nor has it proved necessary. Mechanistically, the physiological bases to explain why 5%–15% of men do not ever suppress sperm output to the desired goal of <1 million/mL, are unknown. It could be that some men have low basal levels of gonadotropin independent spermatogenesis. Alternatively, the administration of exogenous testosterone itself could raise intratesticular testosterone by advection or diffusion sufficiently to maintain a low level of spermatogenesis in some men. In fact, these very low concentrations of intratesticular testosterone are able to maintain spermatogenesis in rodents and non-human primates; and there is no definable dose of testosterone that would both maintain sexual function and suppress gonadotropins without also activating spermatogenesis in rodents. Indirect evidence in humans supports this contention since the use of higher effective testosterone doses as part of MHC results in a greater proportion of men maintaining a low level of spermatogenesis (i.e., less complete suppression of sperm output) (Liu et al., 2008). However, intratesticular testosterone concentrations have not been consistently different between men who respond with lower or higher sperm output while taking MHC (Roth et al., 2016). Nevertheless, using the lowest effective dose of testosterone is advisable to reduce the theoretical risk of breakthrough spermatogenesis, and decrease androgenic side effects such as acne and male pattern balding.

Acceptability of MHC by Couples

Surveys of actual self-reported behaviors show that the male partner is solely responsible for the couple's contraceptive needs in a large proportion, indicating that women can and do trust their partners to share the responsibility to space and time pregnancies. Consistent with this finding, women surveyed concerning their attitudes to a theoretical MHC also indicate that this would be acceptable to them, provided it was a decision shared with their male partner, not with any man in general (Wang et al., 2016). At least a quarter of men, and in some cases many more, would be willing to use male hormonal contraception. These data together suggest that MHC would be widely used by men in cooperation with their female partners, if and when such methods become available. Furthermore, these surveys of a theoretical MHC were conducted in many large cities in Asia, North and South America, Europe and Africa, and indicate that MHC is acceptable by many couples from very diverse backgrounds throughout the world. However, marked geographical variability in theoretical acceptability was apparent, and is consistent with data of actual usage of existing male-directed methods (male sterilization, condom, withdrawal) discussed earlier. Understanding the cultural, religious or other attitudes that underpin this variability may inform strategies to improve usage of male-directed methods in general. Societal changes in attitudes as well as drug development may therefore both be needed to increase usage.

Men participating in MHC studies report that intramuscular injections every 1–3 months, subcutaneous implants every 3 months, or transdermal gels applied every day are acceptable contraceptive methods; however the reports may be biased by selection for, and active participation in, a clinical trial. Reliably understanding the factors that influence acceptability of MHC may therefore require such methods to first become available. Surveys of men contemplating the use of a theoretical MHC product indicate greater acceptability with oral mode of delivery, and higher income and education. However, female-directed methods that require daily dosing are less reliable due to irregular compliance, compared with long-term injectable and implantable contraceptive options. Male-directed methods that also require daily dosing (such as oral or transdermal delivery) are therefore also likely to be less reliable with typical, rather than perfect use. Nevertheless, data from potential users, and actual participants in clinical trials of specific MHC, both indicate that MHC is acceptable to many men and their partners. Multiple MHC treatment options, as exists for female-directed methods, need to be developed to broaden choice.

Effectiveness of MHC

The contraceptive effectiveness of MHC is highly predictable and varies depending on the threshold sperm output achieved, and how consistently suppression to this threshold can be maintained. Mechanistically, this contrasts greatly with hormonal methods in women which either prevent, or do not prevent, ovulation during each menstrual cycle. Men have been exposed to over 3000 person years of androgens with or without progestins, administered to determine the effect on sperm output, or on contraceptive failure rates. Although androgen-progestin drug combinations account for less than one-fifth of the total exposure, many different drug combinations have already been evaluated. This is because the specific delivery, dose and drug combination influences the rate and extent of spermatogenic suppression, which must be individually quantified in order to plan for a large scale contraceptive efficacy study (Liu et al., 2008). To date, only testosterone, or combinations of testosterone with a progestin, have been evaluated to determine contraceptive failure rates (**Table 1**). However, two novel synthetic androgens, 7-alpha-methyl-19-nortestosterone and dimethandrolone (7alpha, 11beta-methyl-19-nortestosterone), have undergone preliminary testing of formulation and dose in humans, with suppression of sperm output as the surrogate endpoint. Both androgens are considerably more potent than testosterone, dimethandrolone also exhibits progestational activity, but cannot be aromatized, whereas 7-alpha-methyl-19-nortestosterone lacks progestational activity, is aromatized, but is resistant to 5-alpha reduction. These biochemical characteristics may allow for the development of a single oral agent with both androgenic and progestational activity (dimethandrolone), or a more favorable risk profile that spares the prostate from overandrogenization by preventing 5-alpha reduction (7-alpha-methyl-19-nortestosterone). Other synthetic androgens could also be developed with specific properties designed to increase effectiveness or limit adverse effects.

Weekly supraphysiological testosterone therapy is a highly effective contraceptive (i.e., prevents pregnancies) and profoundly suppresses spermatogenesis through negative feedback inhibition of gonadotropins (WHO Task Force on Methods for the Regulation of Male Fertility, 1990, 1996; **Table 1**). Longer-acting testosterone formulations administered alone (McLachlan et al., 2000; Gu et al., 2003, 2009) or in combination with either depot medroxyprogesterone acetate (DMPA) (Turner et al., 2003) or norethisterone enanthate (NET-EN) (Behre et al., 2016) are also highly effective (**Table 1**). Individual large scale studies show contraceptive failure rates of 0.8%–2.3%, with upper 95% confidence limits of 1.8%–5.8% (**Table 1**). However, some of these studies did not target sperm concentration to <1 million/mL, and hence may not be applicable for future MHC development.

An integrated analysis of individual participant data of all then-available studies report that 50% and 85% of men suppress sperm output to concentrations compatible with reliable contraception (<1 million/mL), by 3 and 6 months, respectively (Liu et al., 2008). However, this analysis included many exploratory MHC regimens where drug dose and frequency had not yet been optimized. More realistic estimates would be obtained by only examining contraceptive efficacy studies, which are usually extensively tested and optimized. These show that 80%–95% of men suppress sperm output to a threshold of 1 million/mL by 3 months (**Table 1**). The timeframe for this reduction in sperm output compares favorably with the disappearance of sperm after vasectomy.

Progestin co-administration enhances both the rate and extent of sperm output suppression by up to two-fold (Liu et al., 2008). Certain progestins are likely to be more effective in suppressing sperm output than others, due to differences in binding and

activation of progesterone and other steroid receptors. Pharmacokinetic differences are known to explain variation in anovulatory potency and the ability to support pregnancy in women. The hope is that a fully optimized androgen-progestin combination could be utilized by all men within a practical timeframe. To date however, at least 5% of men enrolled in MHC trials never suppress spermatogenesis to levels required for reliable contraception, for mechanistic reasons that are not entirely clear. Another limitation of the method is that sperm rebound, which occurs when sperm suppression below the target threshold is not maintained, is observed in approximately 2% of men. Specifically, 20 (1.7%) of 1193 men who initially suppressed sperm output to below the target of 1 million/mL experienced sperm rebound (**Table 1**). There have not been systematic attempts to determine the characteristics of this group of men, and the mechanisms underlying sperm rebound are not known. Sperm rebound is analogous to breakthrough ovulation that can sometimes occur in women, particularly when using lower dose hormonal contraceptive methods. In men, it cannot be solely explained by poor compliance since study personnel administered MHC in many of these studies.

It is also possible that MHC may not universally suppress sperm output adequately in all men, even after extensive drug and dose optimization. Being able to predict non-suppression for any given drug and dose combination used for MHC would then be important. Systematic analyses show that Caucasian ethnicity and higher BMI are important predictors of non-suppression, although the effect of BMI is small and may not be clinically relevant (Liu et al., 2008). Slower spermatogenic suppression occurs in Asian men, and also with older age, higher baseline testosterone and higher initial sperm concentration, but the independent effect sizes of these latter parameters are small. The large and differing effects of ethnicity on the rate and extent of spermatogenic suppression observed between Caucasian and Asian men may eventually be explained by pharmacogenetic differences, which may ultimately unveil more specific methods to identify the small number of men who do not suppress sperm output adequately.

Reversibility of MHC

An integrated analysis of individual participant data of all then-available studies demonstrated that it is realistic to expect full recovery of spermatogenesis to levels consistent with normal male fertility for all men ceasing male hormonal contraceptives (Liu et al., 2006). At the time, normal male fertility was defined by a sperm concentration of at least 20 million/mL, although sperm concentrations of only 13–15 million/mL are now known to be sufficient, based on an analysis published in 2010 (Cooper et al., 2010). Recovery of sperm output to concentrations consistent with normal male fertility occurs in more than 50% of all men after 6 months, and over 90% of all men by 12 months. The remaining men, if not all men who should recover, do so within 24 months. Older age, Asian ethnicity, lower baseline LH concentration, higher initial sperm concentration and faster initial suppression of sperm output predicted faster recovery. Amongst treatment-related factors, shorter treatment duration and the use of shorter-acting testosterone formulations were associated with faster recovery. Treatment duration had the largest clinically important effect.

Since the publication of the integrated analysis, three large studies, including two contraceptive efficacy studies (Gu et al., 2009; Behre et al., 2016), have been completed (Gu et al., 2009; Mommers et al., 2008; Behre et al., 2016). The first study was conducted in China (Gu et al., 2009), and reported a median time of 7.6 months for sperm output to recover to 20 million/mL (Gu et al., 2009). This is considerably slower than the median recovery time of 3.4 (95%CI 3.2–3.5) months calculated from all previous studies, particularly since faster recovery would have been anticipated for this Asian population (Liu et al., 2006). However, the slower recovery was likely explained by the longer treatment duration of up to 30 months, compared with 12–18 months utilized in all other studies (**Table 2**). The timeframe for recovery of sperm output following cessation of MHC in this study was also consistent with the previous analyses: all except 17 men had recovered by 12 months, and by 15 months one man had a sperm concentration of 13 M/mL (and was then not followed up) and the other presumably never recovered due to intercurrent epididymitis (Gu et al., 2009). The second study was conducted in Europe (Mommers et al., 2008), and 354 men were randomized to receive placebo (53 men) or various regimens of testosterone and the progestin, etonogestrel, to assess suppression of sperm output. The use of a placebo was possible because sperm output, not contraceptive efficacy (i.e., pregnancy rates) was being assessed. Men were treated for 42 or 44 weeks, and amongst those who received active therapy, the median time to recover to a sperm concentration of 20 million/mL was 3.5 months, and by 16 months all men completing follow up had recovered. These data are also compatible with the earlier integrated analysis. Remarkably, two or three men treated with placebo had sperm concentrations <20 million/mL at every assessment during the 24 week follow-up, and up to 14 men (28% of all men treated with placebo) did not consistently maintain sperm concentrations above 20 million/mL during the recovery period. These data from men treated with placebo indicates that supposed non-recovery of sperm output documented in a small number of men treated with MCH could represent regression to the mean, the occurrence of an intercurrent partially sterilizing illness, or both.

The final study was sponsored by the World Health Organization and was conducted in Europe, Asia, South America, and Australia (Behre et al., 2016). Men were treated with a testosterone-progestin combination for up to 18 months, 266 men suppressed sperm output to <1 million/mL, and 96 of these men were discontinued because the study was prematurely terminated (see next section on adverse events). Unlike any of the earlier contraceptive efficacy studies, normal male fertility was defined by a sperm concentration of 15 million/mL or a total sperm count of 39 million per ejaculate, since contemporaneous analyses had established this lower threshold to be compatible with normal male fertility (Cooper et al., 2010). The men treated in this study therefore had a lower initial sperm concentration compared with all previous studies. Interestingly, recovery was slightly delayed, with the median time to recovery to this lower threshold being approximately 6.5 months. This delay is consistent with the earlier integrated analysis which showed slower recovery to either a sperm concentration of either 10 or 20 million/mL, with lower initial sperm concentration. The extent of recovery was also compatible with the earlier integrated analyses since all participants, except 8, recovered by 12 months, and another 5 by 18 months. One participant did not recover even after 4 years, but sperm concentrations were not

Table 2 Pregnancy outcomes of MHC

	Pregnancies													*Recovery of sperm output*	
		Pregnancy outcome						*Pregnancy outcome*							
References	*Pregnancies during treatment* (n)	*LB*	*SA*	*IA*	*UK*	*CM*	*Pregnancies after treatment* (n)	*LB*	*SA*	*IA*	*UK*	*CM*	*Known pregnancies during and after treatment* (n)	*Maximum treatment duration (months)*	*Median time to recovery 20 M/mL (months)*
[a]Gu et al. (2009)	28	0	0	0	28	0	Not reported	–	–	–	–	–	28	30	7.6
[b]WHO (1996)	19	10	4	5	0	0	33	25	1	4	3	0	52	18	2.3
[c]Behre et al. (2016)	4	3	0	1	0	0	Not reported	–	–	–	–	–	4	18	6.5[d]
[a]Gu et al. (2003)	4	0	0	0	4	0	3	0	0	0	3	0	7	12	2–3
[b]WHO (1990)	10	3	0	6	1	0	10[e]	4	1	2	3	0	20	18	3.7
[f]Turner et al. (2003)	0	0	0	0	0	0	5	3	1	0		1[g]	5	18	5.0
[h]McLachlan et al. (2000)	2	2	0	0	0	0	Not reported	–	–	–	–	–	2	18	Approx. 7

LB, live birth; *SA*, spontaneous abortion; *IA*, induced abortions; *UK*, unknown; *CM*, congenital malformation.

[a]Testosterone undecanoate 500 mg/month (with 1000 mg loading dose).

[b]Testosterone enanthate 200 mg/week.

[c]Testosterone undecanoate 1000 mg and norethisterone enanthate 200 mg every 8 weeks.

[d]Recovery to 15 M/mL, not 20 M/mL.

[e]Does not include one pregnancy by a man other than the partner.

[f]Testosterone implant 800 mg every 4 or 6 months with depot medroxyprogesterone acetate 300 mg every 3 months.

[g]One of the twins was born with Vater Anomalad, which was thought to be unrelated to the study drug.

[h]Testosterone implant 800 mg or 1200 mg every 3 months with or without finasteride.

reported, so it is not known whether he remained severely oligozoospermic which could suggest an intercurrent sterilizing disorder, or had values that were closer to 13–15 million/mL which could suggest regression to the mean.

Together these new data from three large trials verify the earlier analyses that concluded that it is reasonable to expect full recovery of spermatogenesis to levels consistent with normal male fertility, and that the rate of recovery is dependent upon treatment duration and initial sperm concentration. Documented non-recovery of sperm output has been reported in only three men from over 2000 men enrolled in MHC trials (Turner et al., 2003; Gu et al., 2009; Behre et al., 2016). Of these three men, an intercurrent and unrelated sterilizing process, rather than any lasting adverse effect of MHC, was more likely to be the true cause: myotonic dystrophy in one man (Turner et al., 2003) and bilateral epididymitis in another (Gu et al., 2009). In the third man, important details regarding his final sperm concentration were not reported (Behre et al., 2016). Important limitations are that few data of MHC are available for men of African or Hispanic origin, or in subfertile men. Another limitation is that systematic studies of sperm recovery after long-term treatment of eugonadal men with androgens or androgen-progestins for contraceptive or non-contraceptive purposes are not available. Case reports do however suggest that sperm quality tends to recover spontaneously within 4–12 months of cessation of high dose androgens in long-term anabolic steroid abusers.

Actual pregnancies and live births have also been observed in couples after cessation of MHC. Specifically, all couples known to be actively seeking parenthood have reportedly been able to do so, and 51 pregnancies resulting in 32 live births have been identified (**Table 2**). A limitation of this analysis is that many couples were likely using other forms of contraception after cessation of MHC if parenthood was still not desired, and long term follow up of subsequent fertility was not systematically obtained.

Safety of MHC

Pregnancy outcomes occurring during or after MHC treatment suggest no increase in fetal miscarriage rates or congenital malformations (**Table 2**). Altogether, 118 pregnancies were reported which resulted in 50 live births, 7 spontaneous abortions, 18 induced abortions, and 1 congenital malformation. Fetal outcomes from the remaining 42 pregnancies were not reported. This corresponds with spontaneous miscarriage rates of 6% (95%CI 2%–12%) assuming no miscarriages amongst pregnancies with unknown outcomes. These miscarriage rates overlap with spontaneous abortion rates of 8%–20% in the general population. The aggregate congenital malformation rate was 0.9% (95%CI 0.0%–4.6%) assuming no malformations amongst pregnancies with unknown outcomes. Accordingly, these data are also consistent with, but are insufficiently powered to exclude the possibility of an increase over the 4% congenital malformation rate in the general population.

A recent methodological advance has been to conduct a randomized placebo controlled androgen-progestin MHC trial from which short-term adverse events due to active treatment could be defined for the first time (Mommers et al., 2008). Almost all men on either active (93%) or placebo (81%) therapy self-reported at least one adverse event, which was mild in severity in almost all cases. However, men receiving active treatment complained twice as frequently than those receiving placebo for certain adverse events: 20% versus 8%, on average for mood and libidinal changes, weight gain, acne and night sweats. These complaints were statistically more prevalent in those treated with active, were generally mild but occasionally led to subject discontinuation (Mommers et al., 2008). Many factors, particularly biochemical factors, were considered clinically irrelevant. Total and high density lipoprotein cholesterol both fell by about 10%, and none of the lipid changes in any treatment or placebo group were statistically significant.

Despite or possibly because of these findings, a recent androgen-progestin MHC efficacy study was prematurely stopped due to concerns regarding mood changes, depression, pain at injection site and increased libido, even though the independent data and safety monitoring board charged with directly overseeing this study found that all criteria for continuation had been met after reviewing the same adverse event data (Behre et al., 2016). Most of these adverse events were mild in severity, only 20 men actually discontinued due to these complaints, and to the contrary, participants themselves reported high levels of satisfaction with the method. Self-reported mood changes and depression in particular, were highly variable across study sites, suggesting interaction with non-drug related factors. Furthermore, depression and mood were only self-reported, and not systematically verified using validated instruments.

In women, the mood changes that occur with estrogen-progestin contraceptives have been attributed to the progestin, and this could also be true for the mood changes reported in both of these androgen-progestin MHC studies. Activation of the gamma-aminobutyric acid type A receptor, which is believed to be important for these mood changes, is also different depending on specific progestins and how they are metabolized. Accordingly, certain progestins could have minimal or no mood problems, but this requires verification. Ultimately, postmarketing surveillance of specific androgen-progestin regimens will be required to properly assess infrequently occurring adverse events. Determining long-term adverse cardiovascular or prostate effects of MHC will also likely require phase four studies since MHC will be most used by younger men in whom the background incidence of either cardiovascular or prostate disease is very low (Piotrowska et al., 2016). It may be that some couples will be willing to accept the theoretical risk of MHC, if the benefits of such therapy in the context of all available options are well-defined. In older men with age-related partial androgen deficiency, for example, lingering concerns regarding the cardiovascular or prostate safety of testosterone therapy remain, yet many men choose to be treated.

Non-hormonal Male Contraception

In contrast to hormonal methods where large-scale multicenter pivotal trials necessary for drug registration by the Food and Drug Administration in the United States are due to start by 2018, the majority of potential non-hormonal male-directed methods are yet

to be tested in humans (Page et al., 2008). Nevertheless, promising targets in the testis, epididymis, sperm, and sperm-egg interaction that appear to be specific and necessary for spermatogenesis, and/or fertilization have been identified. Drugs that can act reversibly and solely on these targets should have few or no systemic adverse effects if these targets are truly specific to spermatogenesis. Despite promising nonclinical preliminary work, all of the few compounds tested in humans thus far have not met these criteria, thereby illustrating the challenges inherent in developing a non-hormonal contraceptive. Gossypol and triptolide, for example, resulted in irreversible infertility. The bis-(dichloroacetyl)-diamines that have been developed thus far to inhibit aldehyde dehydrogenase 1A2, have also inhibited liver aldehyde dehydrogenase thereby causing unacceptable disulfiram-like reactions when co-administered with alcohol. Aldehyde dehydrogenase 1A2 is specific to the testis, and crucial for the conversion of retinaldehyde to retinoic acid, and blocking retinoic acid production in the testis prevents spermatogonial differentiation through suppression of STRA8. The challenge has been developing compounds that specifically inhibit the Aldehyde dehydrogenase 1A2 isoform present only in the testis. A fourth compound, miglustat, induced reversible infertility by inhibiting glycosphingolipid biosynthesis and sperm motility in mice, but miglustat did not alter spermatogenesis in humans. This illustrates that species differences in spermatogenesis exist, and further complicates the development of specific, reversible nonhormonal methods in humans.

Promising testicular targets include bromodomain testis-specific protein-1, which is critical for chromatin remodeling during spermatogenesis, and retinoic acid receptors, activation of which is required for spermatogonial differentiation. Epididymal targets include the epididymal G-protein coupled receptor HE6, which is responsible for reabsorption of tubular fluid, and therefore the sperm microenvironment. Other sperm targets include: epididymal protease inhibitor (EPPIN) which prevents binding of semenogelin 1, a semen coagulation protein, and therefore prevents impaired sperm motility, and; voltage-gated calcium-permeable ion channels (CatSpers), which are important for sperm hyperactivation and motility.

Developing drugs that act on these or other targets would eventually require scalable drug synthesis, stability, and microbial testing, and certification of good manufacturing practice standards. Academic researchers are ill-equipped to meet the regulatory hurdles required for such an undertaking, and hence the National Institute of Child Health and Development has established the male Contraceptive Clinical Trials Network to implement late phase 2 and phase 3 studies for drug registration purposes, and assist in partnering with pharmaceutical industry. Currently this network has focused on hormonal methods. However, the extensive clinical trial experience developed for male hormonal contraceptive methods will be applicable for the testing of non-hormonal approaches, once suitable phase 1 and 2 studies of compounds directed to these targets have been performed.

Summary and Conclusions

Male-directed contraceptive methods are estimated to be worth 40–200 billion dollars worldwide, assuming a market size of 10 million in United States and 50 million worldwide. Many couples already depend upon less than ideal male-directed methods, and there is large unmet need to develop better methods. Despite this, many hurdles remain. Despite this, MHC is the closest to regulatory approval from a drug registration perspective. As discussed, much progress has been made in delineating the effectiveness, reversibility and short-term safety of MHC. Remaining uncertainties include whether such methods can be universally used by all men, how to prevent, predict or monitor sperm rebound, and whether clinically relevant and diagnosable mood changes actually occur, and if it does occur, whether these can be minimized with progestins with optimal characteristics. Fully characterizing the long-term prostate and cardiovascular safety will require post-marketing surveillance, but the low incidence of these disorders in young men provides some reassurance that the absolute risk with MHC is likely to be small. No single female or male directed method will be ideal for all couples at all times, and hence the priority should be to broaden choice to reduce unintended pregnancies, and the individual, family, and societal sequelae that are associated with unintended pregnancies. Male-directed hormonal and non-hormonal methods promise to allow both partners to share equally in family planning responsibilities.

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"Systems Biology of Reproduction"

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Co-Instructor – Eric Nilsson, Abelson Hall 507, 225-1835, nilsson@wsu.edu
Learning Objective -
Current literature based course on the Systems Biology of Reproduction. Learning Systems approaches to the biology of reproduction from a molecular to physiological level of understanding.
Schedule/Lecture Outline –

January	11 & 13	Week 1	Systems Biology Introduction
	18 & 20	Week 2	Molecular/ Cellular/ Reproduction Systems
	25 & 27	Week 3	Sex Determination Systems
February	1 & 3	Week 4	Male Reproductive Tract Development & Function
	8 & 10	Week 5	Female Reproductive Tract Development & Function
	15 & 17	Week 6	Gonadal Developmental Systems Biology
	22 & 24	Week 7	Testis Systems Biology
March	1 & 3	Week 8	Ovary Systems Biology
	8 & 10	Week 9	Epigenetics and Transgenerational Gonadal Disease
	14 – 18	**Week 10**	**Spring Break**
	22 & 24	Week 11	Gametogenesis/ Stem Cells/ Cloning
	29 & 31	Week 12	Hypothalamus-Pituitary Development & Function
April	5 & 7	Week 13	Reproductive Endocrinology Systems
	12 & 14	Week 14	Fertilization & Implantation Systems
	19 & 21	Week 15	Fetal Development & Birth Systems
	26 & 28	Week 16	Assisted Reproduction/Contraception
May	3 & 5	Week 17	Exam or Grant Review

Spring 2022 – Systems Biology of Reproduction
Lecture Outline – Assisted Reproduction/Contraception
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
April 26, 2022
Week 16

Assisted Reproduction/Contraception

Assisted Reproduction –
- Infertility
- ART – Artificial Insemination and Other Technology
- IVF and ICSI
- Pre-implantation Diagnosis (PGD) and Cloning
- Stem Cells and Reproduction

Contraception
- Population Growth and Projections
- Need and History of Contraception
- Sterilization and Other Procedure Use
- Female Hormonal Contraception and Morning After Pill
- Female Implants
- Male Contraception and Targets
- Male Hormonal Contraception and Efficiency
- Other Male Contraceptive Targets

Required Reading

Pennings G, Segers S, Mertes H. (2018) Modern Ethical Dilemmas in ART. in: Encyclopedia of Reproduction 2nd Edition, Ed: MK Skinner. Elsevier. Vol. 5:391-394.
Duffy DM and Archer DF (2018) Female Contraception. in: Encyclopedia of Reproduction 2nd Edition, Ed: MK Skinner. Elsevier. Vol. 2:255-260.
Liu PY (2018) Male contraception. in: Encyclopedia of Reproduction 2nd Edition, Ed: MK Skinner. Elsevier. Vol. 1:478-485.

Spring 2022 – Systems Biology of Reproduction
Discussion Outline – Assisted Reproduction/Contraception
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
April 28, 2022
Week 16

Assisted Reproduction/Contraception

Primary Papers:

1. Oritz-Rodriguez, et al. (2019) Plos One 0213420, 1-24.
2. Shetty, et al. (2021) Andrology 9:1603
3. Chang, et al. (2021) Nature Communications 12:1253

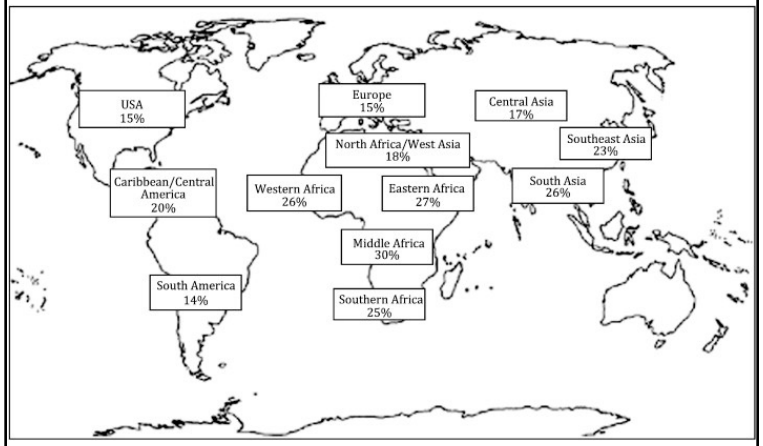
Discussion

Student 12: Reference 1 above
- What is the assisted reproductive technology (ART) investigated?
- What is the experimental design and technology used?
- What is the conclusion on the use of this ART technology?

Student 18: Reference 2 above
- What is spermatogonial transplantation?
- What was the experimental design?
- What applications does the technology have?

Student 20: Reference 3 above
- What is the compound and where is it from?
- What was the experimental design?
- Is this a contraceptive and what clinical issues should be investigated?

The changing prevalence of infertility.
Petraglia F, Serour GI, Chapron C.
Int J Gynaecol Obstet. 2013 Dec;123 Suppl 2:S4-8.



Percentage of women aged 25 through 49 years with secondary infecundity, by region, 1994–2000 (according to data collected from WHO website).

Table 16.5 lists the more common categories of dysfunction that lead to infertility in women. It is very difficult to give precise values to the relative incidence of each of

Table 16.5
Major Causes of Infertility in Women

Cause	Examples of disorders
Ovulatory failure	Polycystic ovarian syndrome Hypothalamic anovulation Gonadal dysgenesis Resistant ovarian syndrome Hypogonadotropic hypogonadism
Impaired gamete or zygote transport	Impaired oocyte capture Uterine tube cilia defects Uterine tube defects Endometriosis Antisperm antibodies Pelvic inflammatory disease Cervical mucus
Implantation defects and recurrent spontaneous abortion	Polycystic ovarian syndrome Chromosome abnormalities Uterine and endometrial anomalies Corpus luteum defects

Adapted from Healy *et al.*, 1994, with permission.

Table 16.6
Infertility in Males

Conditions resulting in untreatable sterility	Seminiferous tube failure Chromosome disorders—Klinefelter syndrome Cryptorchidism Testicular atrophy Idiopathic
Potentially treatable conditions	Genital tract obstructions Congenital STDs or other types of infections Trauma Sperm autoimmunity Idiopathic Trauma Gonadotropin deficiency Hypothalamic dysfunction Pituitary disorders Hyperprolactinemia Coital disorders Impotence Retrograde ejaculation Failure of ejaculation
Untreatable subfertility	Oligospermia—sperm count less than 20 million/ml Asthenospermia—less than 25% motile sperm Teratospermia—greater than 85% abnormal sperm Nomospermia—normal-looking sperm, but with impaired ability to fertilize

Adapted from Baker, 1994, with permission.

Assisted Reproductive Technologies (ART)

-In Vitro Fertilization (IVF)

-Intracytoplasmic Sperm Injection (ICSI)

-Nuclear Transfer

-Embryo Transfer

-Cloning

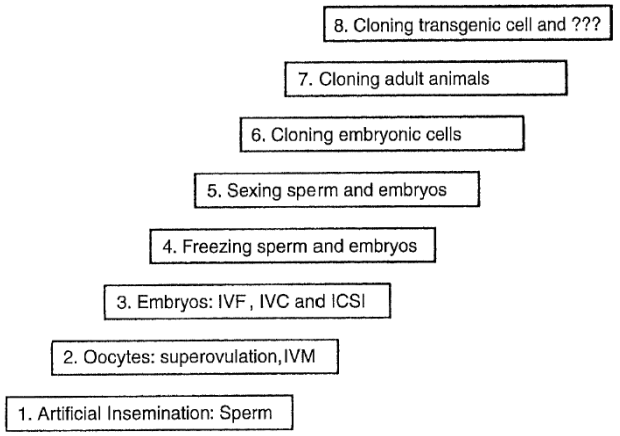


FIG. 1. Reproductive biotechnology ladder, starting with artificial insemination and proceeding somewhat chronologically and technologically up the ladder. IVM = *in vitro* maturation; IVF = *in vitro* fertilization; IVC = *in vitro* culture; ICSI = intra-cytoplasmic sperm injection.

Artificial insemination: the state of the art

R. Vishwanath*

Livestock Improvement Corporation Ltd., Private Bag 3016, Hamilton, New Zealand

Abstract

The history of research into artificial insemination (AI) is over two centuries old and its commercial application now spans 75 years. It is appropriate to reflect on the contribution of this powerful method of gene dispersal. AI remains as one of the most important assisted reproductive technologies. The three cornerstones for its application are: it is simple, economical and successful. The importance of AI will be challenged in the next few decades. The remarkable progress made in other assisted reproductive technologies does have the potential to rapidly generate offspring. The challenge for any of these reproductive technologies to attain widespread use is to match AI in being simple, economical and successful. This review aims at capturing the salient advances in AI, the comparisons with natural mating and other reproductive technologies, and, whether the future of AI will be challenged. It predicts what the new horizon looks like and the role that AI will play in the overall reproductive technologies landscape.

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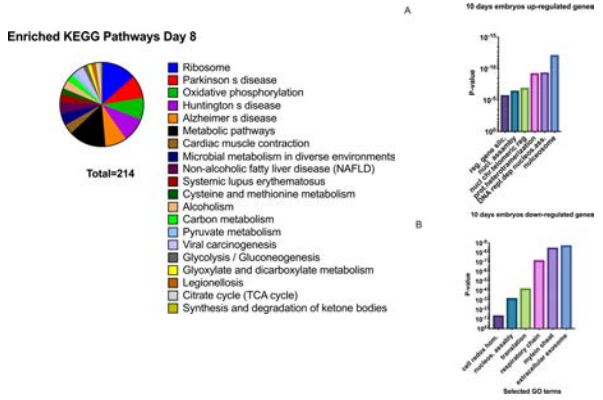
Keywords: Sperm; Storage; Frozen semen; Liquid semen; Artificial insemination

Table 2
Summary of cattle AI statistics with frozen semen from different countries

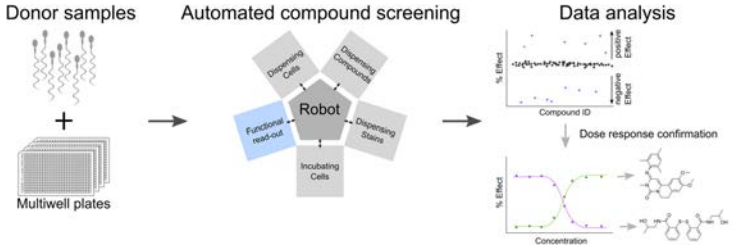
Country	Number of inseminations[a]	Frozen (%)[b]	Number of sperm inseminated	Fertility (%)	Non-return (days)
Austria	915490	100	25×10^6	72.3	59
Australia	1600000	100	20×10^6	60	–
Belgium	581000	100	12×10^6 to 15×10^6	71	56
Brazil	2861852	100	40×10^6	60–70	–
Canada	1500000	100	15×10^6	68	60–90
China	10000000	100	–	70	–
Denmark	787848	100	15×10^6	67.8	56
Finland	462852	100	20×10^6	63.8	60
France	4800000	90	20×10^6	60	48
Germany	5577981	98	10×10^6 to 20×10^6	64.6	60–90
Hungary	400000	100	20×10^6 to 25×10^6	50	Pregnant
Israel	150000	100	20×10^6	55	59
Italy	2450000	100	18×10^6	63.8	59
Japan	2173456	99	20×10^6	58	60–90
Korea	1586000	94	30×10^6	71	59
Mexico	900000	95	35×10^6	40	59
The Netherlands	1659496	100	Varies by bull	69.4	56
New Zealand[c]	3800000	37	1×10^6 to 2×10^6	69	2–24
Norway	419137	100	18×10^6	70.9	60–90
Poland	2175840	100	20×10^6	70	60
Spain	1800000	95	30×10^6	65–70	59
Sweden	480000	100	15×10^6	68	56
Switzerland	1098000	100	20×10^6	68.8	75
USA	10466000	100	10×10^6 to 30×10^6	68	59

[a] Data are based primarily on dairy cattle. In Italy 1500 buffaloes are inseminated with fresh semen in egg yolk-Tris (EYT).
[b] Most semen was frozen in EYT, with yolk-citrate milk, Biociphos, and Trilady also used. Frozen-thawed sperm were reported to be 45–60% motile.
[c] New Zealand uses 1×10^6 to 2×10^6 total sperm for liquid semen in Caprogen® during their restricted breeding season. The average sperm concentration for frozen semen is 10–20 million.

Transcriptome analysis reveals that fertilization with cryopreserved sperm downregulates genes relevant for early embryo development in the horse.
Ortiz-Rodriguez JM1, Ortega-Ferrusola C, Gil MC, et al.
PLoS One. 2019 Jun 25;14(6):e0213420.



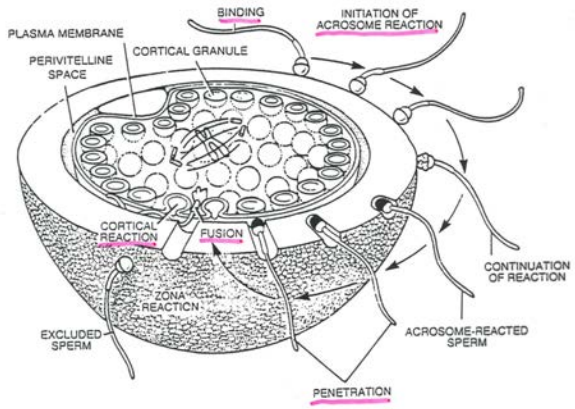
High-throughput phenotypic screening of the human spermatozoon
Johnston ZC, Gruber FS, Brown SG, et al.
Reproduction. 2021 Dec 27;163(1):R1-R9.

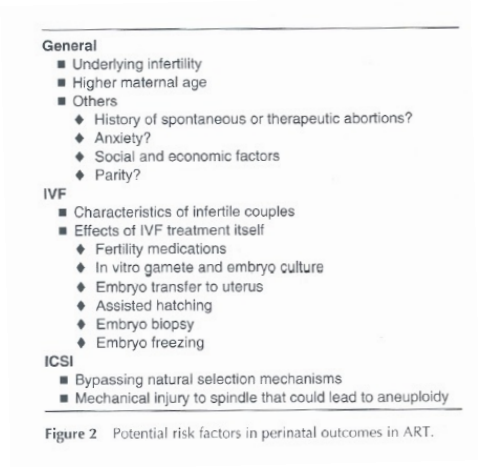


The UK´s anomalous 10-year limit on oocyte storage: time to change the law.
Bowen-Simpkins P, Wang JJ, Ahuja KK.
Reprod Biomed Online. 2018 Oct;37(4):387-389.

Abstract

There has been a growing recognition in the UK that the statutory storage limit for frozen eggs, which currently stands at 10 years, requires a review. The UK regulator, the Human Fertilization and Embryology Authority (HFEA), has recognized the problem and the Equality and Human Rights Commission is also sympathetic with the demand to change the current legislation. There is also strong desire on the part of assisted reproductive technology (ART) professionals and patients to change the current guidelines. For many women, the available alternatives of transporting their eggs to an overseas destination or having them fertilized with donor sperm and then stored as embryos is objectionable.




General
- Underlying infertility
- Higher maternal age
- Others
  - History of spontaneous or therapeutic abortions?
  - Anxiety?
  - Social and economic factors
  - Parity?

IVF
- Characteristics of infertile couples
- Effects of IVF treatment itself
  - Fertility medications
  - In vitro gamete and embryo culture
  - Embryo transfer to uterus
  - Assisted hatching
  - Embryo biopsy
  - Embryo freezing

ICSI
- Bypassing natural selection mechanisms
- Mechanical injury to spindle that could lead to aneuploidy

Figure 2 Potential risk factors in perinatal outcomes in ART.

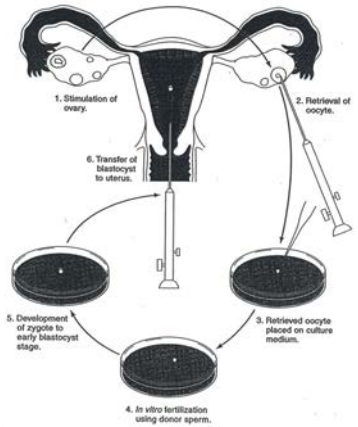
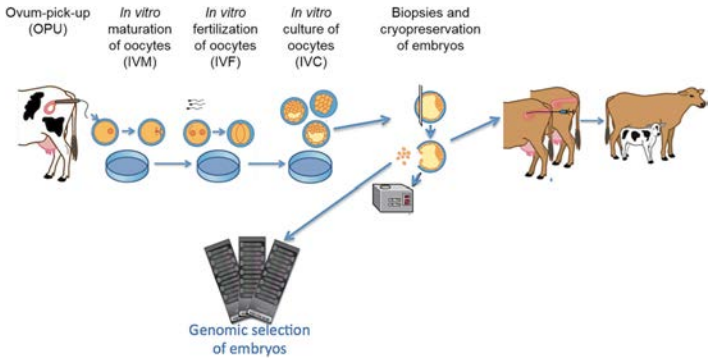


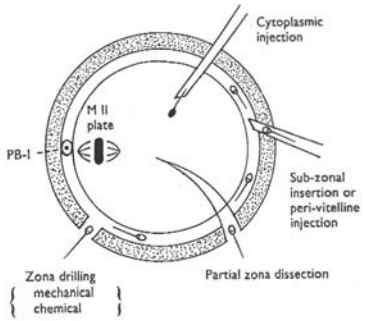
Figure 16.1
Standard IVF and ET protocol.

Review: Recent advances in bovine in vitro embryo production: reproductive biotechnology history and methods.
Ferré LB, Kjelland ME, Strøbech LB, Hyttel P, Mermillod P, Ross PJ.
Animal. 2019 Nov 25:1-14.



ICSI

Figure 1



Microfertilization by various methods.

sively motile. The disadvantage is that this procedure cannot be appli immotile spermatozoa.

Partial zona dissection
The oocyte is held by a pipette and a portion of the zone pellucida is cu a fine glass needle or metal microblade. An advantage of this procedure cytoplasm injection is that one potential sperm selection barrier, the vi membrane, is present as in zona drilling. In this procedure the sperm m capacitated and acrosome-reacted for fusion with a vitelline membra disadvantage of this method is that an abnormal embryo hatching proce been observed after embryo transfer (Talansky & Gordon, 1988; Mal

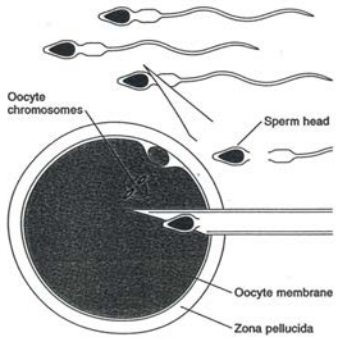
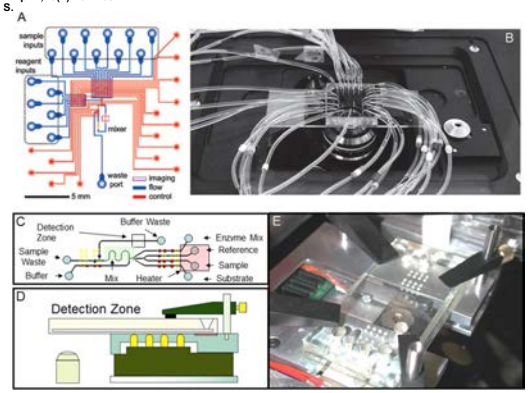


Figure 16.2
ICSI procedure. Removal of the sperm tail by an injection pipette, and injection of an isolated sperm head into the oocyte.

Application of microfluidic technologies to human assisted reproduction.
Mol Hum Reprod. 2017 Apr 1;23(4):257-268.
Smith GD, Takayama S.



Potential risks associated with ICSI

Mild male infertility
- Genetic problems unlikely
- Theoretical adverse effects via
- Spindle damage
  - polyvinylpyrrolidone (PVP) exposure
  - Subtle disordering of normal processes of fertilization

Severe male infertility
- Greater potential for passing on preexisting paternal genetic anomalies (Y deletions or expanded trinucleotide repeats)

Potential risks associated with sperm source

Epididymal sperm
- Screen for cystic fibrosis in congenital absence of vas deferens
- No additional confounding factors

Testicular sperm
- Greater potential of complicating factors
- Immature sperm
- Rare imprinting disorders
- Transmission of severe male infertility from father?
- Severe male genetic problems associated with other genetic anomalies?

A. Van Steirteghem et al. / Molecular and Cellular Endocrinology 186 (2002) 199–203

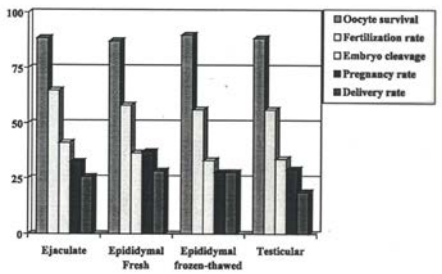


Fig. 2. ICSI results in relation to type of sperm used (adapted from results in Bonduelle et al., 1998)

Table 16.12
Comparison of Reported Outcomes for ART Procedures

Variable	IVF	GIFT	ZIFT	ICSI
Number of procedures	36,035	3741	1078	5052
Transfers per retrieval (%)	89.7	98.5	91.7	92.5
Pregnancies	8299	1142	322	1461
Pregnancy loss (%)	18.6	21.5	18.3	18.9
Deliveries	6754	896	263	1185
Deliveries per retrieval (%)	22.3	27.0	27.9	23.5
Single births (%)	64.1	65.3	68.8	64.1
Ectopic pregnancies per transfer (%)	0.9	1.2	1.4	0.3
Birth defects per birth (%)	0.7	0.3	0.6	1.1

Data from 1995 and compiled from 281 clinics in the United States and Canada. (Adapted from ARTs in 1995, 1998, with permission.)

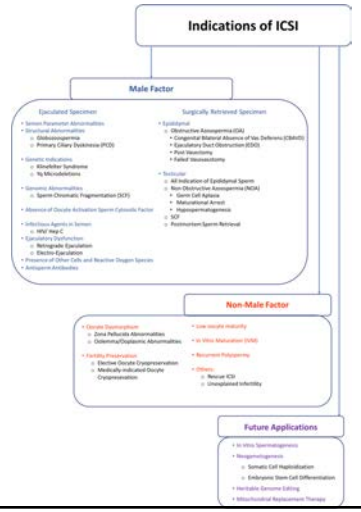
Table 6. Structural congenital abnormalities in births and pregnancies terminated for congenital anomalies in IVF compared with spontaneously conceived pregnancies

	IVF			IVF-ICSI		
Structural abnormality	Incidence in IVF conception %	Incidence in spontaneous conception %	*P*	Incidence in IVF-ICSI conception %	Incidence in spontaneous conception %	Relative risk / Odds ratio / *P*
Any major malformation*	9.0[92]	4.2[92]	< 0.001[92]	8.6[92], 8.8[47]	4.2[92], 6.1[47]	0.001[92], 1.4[47]**
Cardiovascular	1.8[92]	0.6[92]	< 0.001[92]	1.3[92], 2.1[47]	0.6[92], 1.4[47]	> 0.05[92], 1.5[47]**
Gastrointestinal	0.6[92]	0.6[92]	> 0.05[92]	0.7[47], 1.0[92]	0.3[47], 0.6[92]	> 0.05[92], 2.6[47]**
Urogenital	2.6[92]	1.4[92]	0.01[92]	2.3[92], 3.2[47]	1.4[92], 1.5[47]	> 0.05[92], 2.2[47]
Musculoskeletal	3.3[92]	1.1[92]	< 0.001[92]	1.8[47], 3.3[92]	1.1[92], 1.8[47]	0.004[92], 1.0[47]
Central nervous system	0.4[92]	0.2[92]	> 0.05[92]	0[92], 0.6[47]	0.2[92], 0.6[47]	1.0[47]

*As defined by the respective birth registries. Superscripts refer to reference numbers unless otherwise stated. **$P < 0.05$. IVF: in vitro fertilization; ICSI: intracytoplasmic sperm injection; IVF: in vitro fertilization; NICU: neonatal intensive care unit.

Thoughts on the popularity of ICSI.

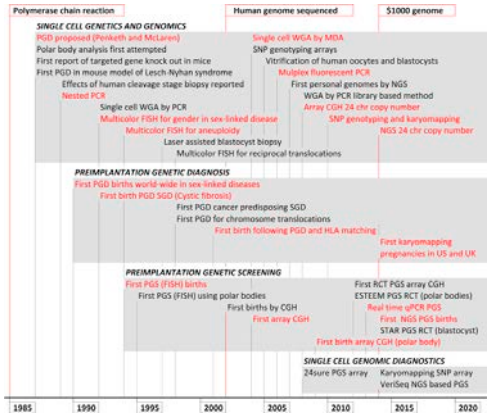
Haddad M, Stewart J, Xie P, Cheung S, Trout A, Keating D, Parrella A, Lawrence S, Rosenwaks Z, Palermo GD. J Assist Reprod Genet. 2021 Jan;38(1):101-123.



PREIMPLANTATION GENETIC DIAGNOSIS

Peter Braude, Susan Pickering, Frances Flinter and Caroline Mackie Ogilvie

Preimplantation genetic diagnosis (PGD) is an evolving technique that provides a practical alternative to prenatal diagnosis and termination of pregnancy for couples who are at substantial risk of transmitting a serious genetic disorder to their offspring. Samples for genetic testing are obtained from oocytes or cleaving embryos after *in vitro* fertilization. Only embryos that are shown to be free of the genetic disorders are made available for replacement in the uterus, in the hope of establishing a pregnancy. PGD has provided unique insights into aspects of reproductive genetics and early human development, but has also raised important new ethical issues about assisted human reproduction.



A brief timeline of preimplantation genetic testing. Some of the main developments in 1) single cell genetics and genomics, 2) preimplantation genetic diagnosis (now preimplantation genetic testing for monogenic disease [PGT-M]), 3) preimplantation genetic screening (now preimplantation genetic testing for aneuploidy [PGT-A]), and 4) commercially available single cell diagnostics (panels from top to bottom) are listed from the middle 1980s onward (see text for some of the key references). There were three major milestones in human molecular genetics over this period: the development of the polymerase chain reaction (PCR) for amplifying short fragments of DNA in the middle 1980s; the sequencing of a first draft of the human genome in the early 2000s, and the first $1,000 whole-genome sequencing in 2014.

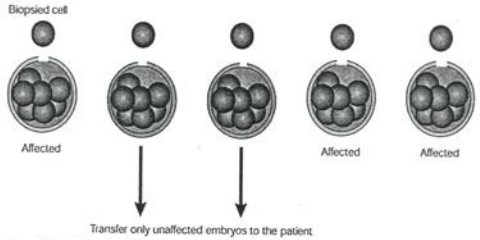
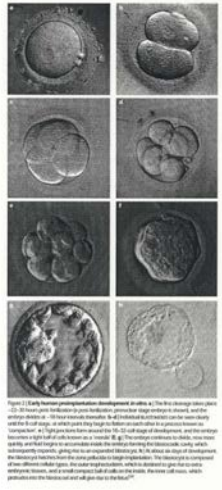


Figure 1 | **Principle of preimplantation genetic diagnosis.** A single cell (or cells) is removed from each embryo of an *in vitro*-developing cohort, on which a diagnostic genetic test is carried out. Up to three of the embryos that are unaffected are transferred to the patient in the hope of establishing a pregnancy.



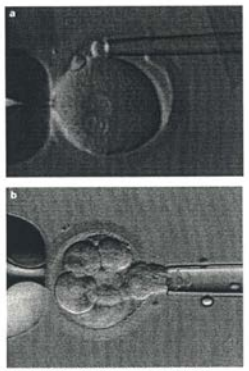


Figure 3 | **Polar body and cleavage stage biopsies.** **a** | Polar body biopsy. Around 14–20 hours after normal fertilization, the zona pellucida of the zygote is breached by partial zona dissection using a microneedle and then a small aspiration capillary is introduced under the zona and the first and second polar bodies removed by gentle suction (reproduced with permission from Reproductive Genetics Institute, Chicago). **b** | Cleavage stage biopsy. Cleavage stage embryos are taken ~ 72 hours post-fertilization and held stationary on a glass micropipettte by gentle suction. The zona pellucida is breached either by laser beam or by a jet of acidified Tyrodes solution. A sampling pipettte is introduced into the embryo and a single nucleated blastomere is removed by suction.

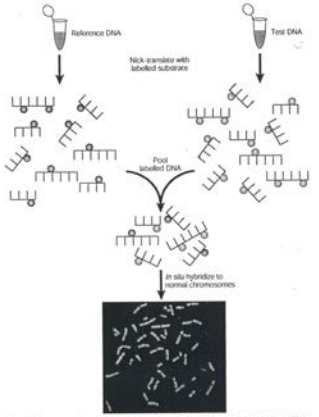
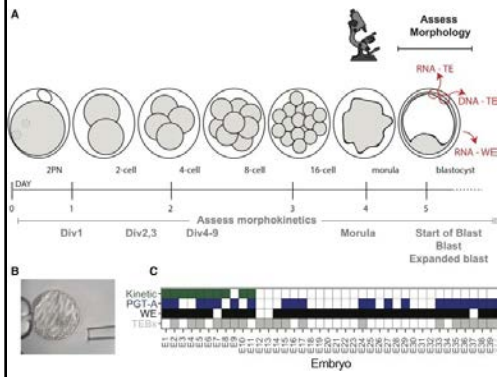


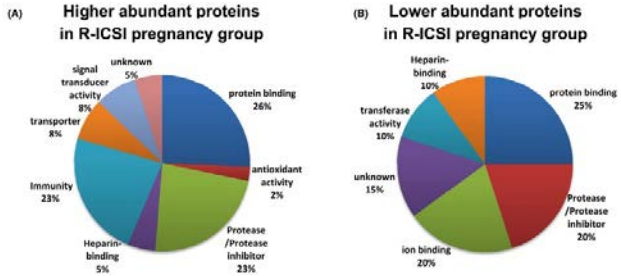
Figure 7 | **Comparative genomic hybridization.** In this technique, reference and test DNA samples are fluorescently labelled in NICK-TRANSLATION reactions. After hybridization of labelled probe mixes to normal chromosome spreads, relative fluorescent intensity is detected by capture of fluorescence using a cooled charge-coupled device camera. Dedicated software is used to compare ratios of green to red fluorescence along each chromosome and, hence, to identify genome imbalance in the test DNA. The figure is based on images supplied by Vysis, Inc. (Downers Grove, Illinois, USA).

RNA-seq as a tool for evaluating human embryo competence.
Groff AF, Resetkova N, DiDomenico F, Sakkas D, Penzias A, Rinn JL, Eggan K.
Genome Res. 2019 Oct;29(10):1705-1718.



Experimental overview. (*A*) Preimplantation human development time-course depicting our comparative analytical approach. Samples were processed from blastocyst stage embryos and assessed for morphokinetic criteria and morphology before biopsy. One trophectoderm (TE) biopsy was processed for DNA-based preimplantation genetic testing for aneuploidy (PGT-A), one was harvested for RNA-seq, and the remaining whole embryo (WE) was also processed for RNA-seq. (*B*) Representative image of a blastocyst. (*C*) Data overview table. Embryos (E1-39) for which we have morphokinetic data are shaded in green; those for which DNA-based PGT-A yielded a result are depicted in blue; and those for which we have RNA-seq of either WE or TE biopsy are labeled in black and gray, respectively.

Characterization of seminal plasma proteomic alterations associated with the IVF and rescue-ICSI pregnancy in assisted reproduction.
Liu X, Liu G, Zhu P, Wang Y, et al,
Andrology. 2020 Mar;8(2):407-420.



New perspectives on preimplantation genetic diagnosis and preimplantation genetic screening.

Chen CK, Yu HT, Soong YK, Lee CL.
Taiwan J Obstet Gynecol. 2014 Jun;53(2):146-50.

Table 1
Differences between the biopsy methods used for preimplantation genetic diagnosis.

Stage of biopsy	Advantages	Disadvantages and limitations
Oocyte/zygote (polar body)	No effect on subsequent embryo development Enough time to perform analysis prior to transfer	Only one or two cells available for analysis Only maternal message obtained DNA liable to degenerate
Cleavage stage	Diagnosis of maternal and paternal inherited disease Possibility of sex determination Up to two cells available for analysis	Limited time for analysis High incidence of chromosomal mosaicism
Blastocyst	Fewer number of embryos to be biopsied and fewer specimens to process Three or more cells per embryo available to overcome allele dropout Less problem of mosaicism	Occasions when embryos failing to blastocysts Need for cryopreservation most of the time

Table 2
The list of common indications for single-gene defects based on information from the ESHRE PGD consortium [40].

Disease
Cystic fibrosis
Myotonic dystrophy
Huntington disease
Beta thalassemia
Fragile X syndrome
Spinal muscular atrophy
Hemophilia A
Duchenne muscular dystrophy/Becker muscular dystrophy
Human leukocyte antigen
Others[a]

Note. From "The causes of misdiagnosis and adverse outcomes in PGD" by L. Wilton, A. Thornhill, J. Traeger-Synodinos, K.D. Sermon, and J.C. Harper, 2009, Hum Reprod, 24, p. 1221–8. The Authors 2009. Reproduced with permission.
[a] Others: sickle cell anemia, neurofibromatosis type 1, tuberous sclerosis, von Hippel Lindau, familial adenomatous polyposis, Charcot–Marie–Tooth disease type 1, Marfan syndrome, familial amyloidotic polyneuropathy.

Strategies to Identify Genetic Variants Causing Infertility
Ding X, Schimenti JC.
Trends Mol Med. 2021 Aug;27(8):792-806.

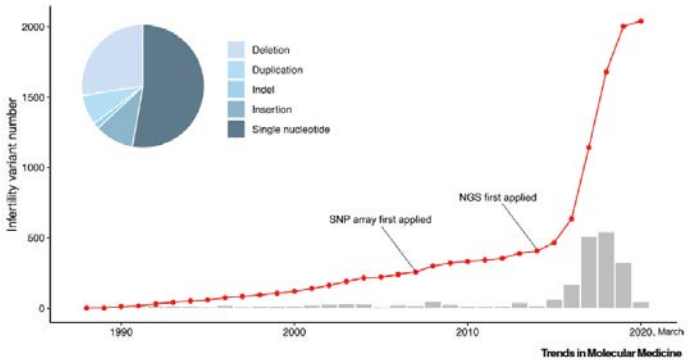


Figure 1. Infertility Variants in the ClinVar Database. Red line, cumulative pathogenic variants in the database. Gray bars, number added annually. The pie chart shows the distribution of variant types. Graphs were made using R. Abbreviation: NGS, next-generation sequencing.

New insights into the genetic basis of infertility.

Venkatesh T, Suresh PS, Tsutsumi R.

Appl Clin Genet. 2014 Dec 1;7:235-43.

Table 1 List of genes examined in female infertility as discussed in the review

	Infertility disorder	Genes	Reference
1	Polycystic ovary syndrome	*StAR, CYP11, CYP17, CYP19 HSD17B1-3, HSD3B1-2, ACTR1, ACTR2A-B, FS, INHA, INHBA-B, INHC, SHBG, LHCGR, FSHR, MADH4, AR, MC4R, OB, OBR, POMC, UCP2-3, IGF1, IGF1R, IGFBP1-3, INS VNTR, IR, INSL, IRS1-2, PPARG*	18
		LHCGR, FSHR	20–27
		VDR	29
		EPHX1, LMNA, GSK3A	32–34
2	XX, gonadal dysgenesis	*FSHR, BMP15, NR5A1, EIF2B2, EIF2B5, HSD17B4, HARS2*	36–39
		PSMC3IP	36
	Perrault syndrome	*HSD17B4*	41
		LARS2, HARS2	42,43
3	Premature ovarian failure	*FSH, FOXO3A, FOXL2, BMP15*	10
		TSHB, ADAMTS16	45
		PCSK1, DBH	46
		FMR1	47

Note: Readers are requested to refer to the cited references for further details regarding the gene association.

Table 2 List of genes examined in male infertility as discussed in the review

	Infertility disorder	Genes	References
1	Leydig cell hypoplasia	*LHCGR*	50,51
2	XY, gonadal dysgenesis	*MAP3K1, SRY, SF1, DHH*	58–60
		SUPT3H, PRKACG, FAM189A2, C2ORF80	61
		DMRT1, MAMLD1	62,63
3	Spermatogenic failure	*RBYM1A1, BPY2, DBX3Y, USP9Y, DAZ1, HSFY1, TSPY1*	64
		CDY2A, HSFY1	73
		TAF4B, SMYD3, DMRT1	75,76
		PIWIL2	77

Note: Readers are requested to refer to the cited references for further details regarding the gene association.

Epigenetic Mechanisms of ART-Related Imprinting Disorders: Lessons From iPSC and Mouse Models

Horánszky A, Becker JL, Zana M, et al.

Genes (Basel). 2021 Oct 26;12(11):1704.

Table 1. DNA methylation and expression alterations of the imprinted genes implicated in IDs after ART procedures in mice.

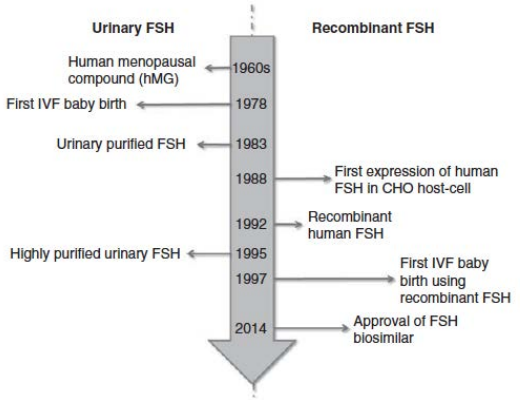
Procedure	Imprinted Gene	Reported Alteration	ID Associated with Imprinted Region	References
Ex vivo embryo culture	*H19*	LOM and biallelic expression LOM at ICR	SRS	[62,63] [56,64]
IVF	*H19*	Aberrant imprint methylation resulting in biallelic expression rather than expression solely from maternal allele.	SRS	[55] [65]
	H19	Aberrant methylation patterns at ICR LOM at ICR GOM at maternal ICR	BWS	[64] [55]
Vitrification	*Grb10* *KvDMR1*	Reduced expression accompanied by downregulation of methylation. Reduced methylation does not explain altered expression. GOM in fetuses compared to in vitro culture samples	SRS BWS	[66] [54]
ICSI	*Snrpn* *Peg3* *H19*	LOM at maternal DMR and aberrant expression LOM at maternal DMR and aberrant expression LOM at ICR	PWS / SRS	[67] [64]

Procedure	Imprinted Gene	Reported Alteration	ID Associated with Imprinted Region	References
Superovulation	*H19*	Aberrant expression Increased expression	SRS	[65] [45]
	Snrpn	LOM at paternal allele	PWS	[44]
	KcnqIot1	LOM at maternal ICR	BWS	[44]
	Gbr10	LOM at DMR LOM at maternal ICR GOM at CG11 and decreased expression	SRS	[68] [44] [45]
In vitro follicle culture	*H19*	LOM at DMR	SRS	[68]
	Snrpn	LOM at DMR	PWSSRS	[68]
	Mest	LOM at DMR		[68]

Biosimilar recombinant follicle stimulating hormones in infertility treatment.

Santi D, Simoni M.

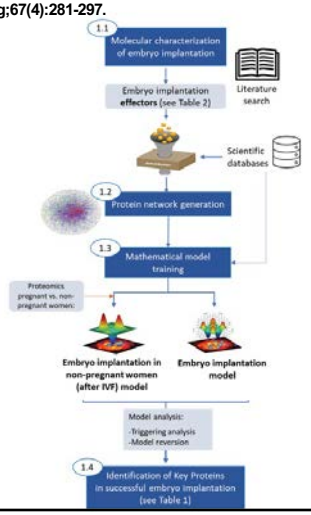
Expert Opin Biol Ther. 2014, 14(10):1399-409.



Proteomics based drug repositioning applied to improve in vitro fertilization implantation: an artificial intelligence model.

Matorras R, Valls R, Azkargorta M, et al.

Syst Biol Reprod Med. 2021 Aug;67(4):281-297.



Drug			Predicted efficacy		Targeted Key proteins	FDA Preg. Categ.	MoA Biblio support
Drug ID	Generic Name	Route of admin.	Implantation Effectors	Key proteins			
DB00065	Infliximab	Intravenous	83 %	76 %	TNF	B	√
DB09221	Polaprezinc	Oral	71 %	69 %	HSP90AB1, HSP90AA1, TNF	NA	√
DB01427	Amrinone	Intravenous, Oral	70 %	58 %	TNF	C	√
DB00636	Clofibrate	Oral	64 %	72 %	PPARA	C	√
DB01241	Gemfibrozil	Oral	64 %	72 %	PPARA	C	√
DB09064	Ciprofibrate	N/A	64 %	72 %	PPARA	C	√
DB04519	Caprylic acid	Oral	64 %	72 %	PPARA	C	√
DB11644	Tafamidis	Oral	62 %	71 %	-	C	√
DB06410	Doxercalciferol	Intravenous, Oral	58 %	73 %	VDR	B	√
DB00146	Calcidiol	Oral	58 %	73 %	VDR	C	√
DB01070	Dihydrotachysterol	Oral	58 %	73 %	VDR	C	√
DB01039	Fenofibrate	Oral	49 %	75 %	PPARA, NR1I2	C	√
DB13873	Fenofibric acid	Oral	49 %	75 %	PPARA, NR1I2	C	√
DB00378	Dydrogesterone	N/A	82 %	67 %	PGR	A	!
DB01431	Allylestrenol	N/A	80 %	73 %	PGR, ESR1	A	!
DB06205	Hyaluronidase (Human recombinant)	Subcutaneous	78 %	43 %	-	C	!
_	Heparinoid	N/A	77 %	71 %	MMP9	NA	!
DB03088	Pidolic Acid	Oral	75 %	70 %	VEGFA	NA	!
DB00098	Antithymocyte immunoglobulin (rabbit)	Intravenous	74 %	52 %	-	C	!
DB00573	Fenoprofen	Oral	74 %	69 %	PPARA	NA	!
DB01065	Melatonin	Oral	73 %	75 %	ESR1, MTNR1A	C	!
DB00744	Zileuton	Oral	72 %	66 %	-	C	!
DB00729	Diphemanil Methylsulfate	N/A	44 %	73 %	-	NA	!

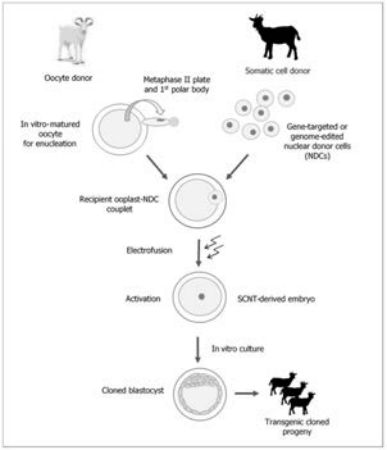
Light blue columns show information about the drug: its identifier in DrugBank Database (Drug ID), its name and its route/s of administration. Those drugs that have been linked to implantation failure prevention in scientific papers are highlighted in gray. Dark blue columns display the predicted efficacy of the drug to modulate the EI proteins effectors (Implantation Effectors) and predicted key proteins (Key proteins) according to the mathematical models. Column 'Targeted Key proteins' shows the targets that have been identified as key proteins in this study. The column 'FDA Pregancy category' shows the 'classical' Pregnancy category associated with the drug by FDA (A, B, C, D, X; NA if information was not found). The column in the right indicates the plausibility of a mechanism of action hypothesis supported by bibliography.

25th ANNIVERSARY OF CLONING BY SOMATIC-CELL NUCLEAR TRANSFER: Cloning, mitochondrial replacement and genome editing: 25 years of ethical debate since Dolly.
Greenfield A.
Reproduction. 2021 Jun 11;162(1):F69-F78.

Abstract

The birth of Dolly the sheep in 1996 elicited a tsunami of commentaries, both in the popular media and academic journals, including responses to the prospect of human reproductive cloning. Much of the anxiety expressed over this imagined consequence of Dolly's genesis revealed fundamental concerns about us losing our commitments to certain ethical goods, such as human dignity, or even 'what it means to be human'. Over the last 25 years, the focus of much of the ethical debate over human biotechnology has slowly shifted towards other genetic technologies that aim to influence inheritance, such as mitochondrial replacement techniques (MRT) and heritable genome editing. Genome editing, in particular, is a technology with multiple fields of application, actual and potential, in research and innovation. This review suggests that many of the fundamental concerns about the possibility of human reproductive cloning that were precipitated by Dolly persist today in the arguments of those who oppose MRT and any use of heritable human genome editing (HHGE). Whilst it is not accepted here that an understanding of human nature and dignity alone can demonstrate the ethical unacceptability of such assisted reproductive technologies, there are themes of justice, which extend into our relationships with animals, that demand continued wide-ranging examination and public dialogue. While Dolly has cast a long shadow over such discussions, this review suggests that the general existential angst over human uses of biotechnology that she came to symbolise is neither compulsory nor a reliable guide for how to think about biotechnologies today.

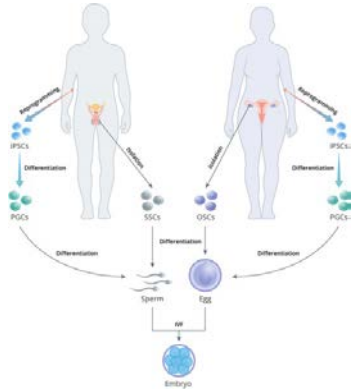
Generating Cloned Goats by Somatic Cell Nuclear Transfer-Molecular Determinants and Application to Transgenics and Biomedicine
Skrzyszowska M, Samiec M.
Int J Mol Sci. 2021 Jul 13;22(14):7490.



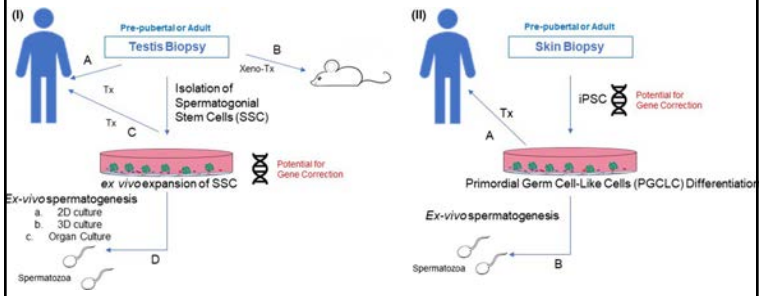
Generation of transgenic cloned goats by somatic cell nuclear transfer (SCNT).

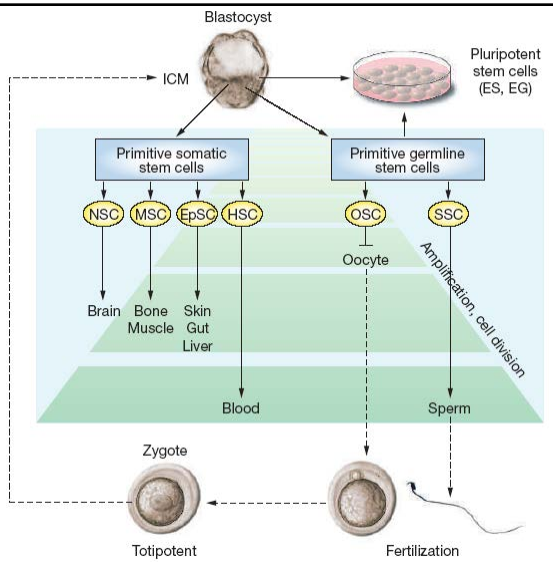
Stem Cells and ART

What can stem cell technology offer to IVF patients?
Ilic D, Telfer EE, Ogilvie C, Kolundzic N, Khalaf Y.
BJOG. 2019 Jun;126(7):824-827.

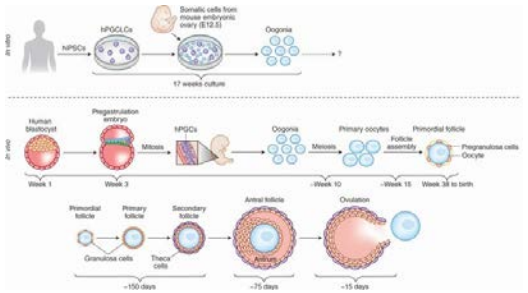


Potential use of stem cells for fertility preservation.
Gauthier-Fisher A, Kauffman A, Librach CL.
Andrology. 2019 Sep 27. doi: 10.1111/andr.12713. [Epub ahead of print] Review.



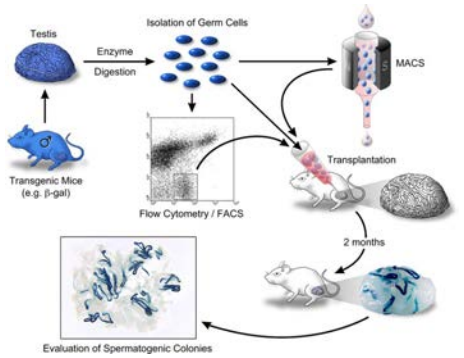


A step toward making human oocytes.
Stringer JM, Western PS.
Nat Biotechnol. 2019 Jan 3;37(1):24-25.



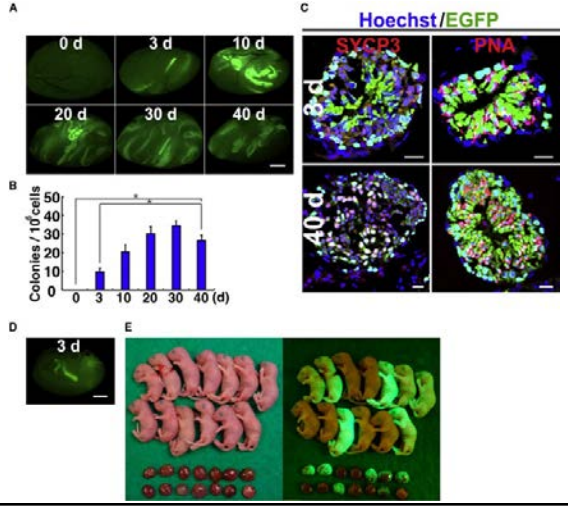
Production of human oogonia–like cells *in vitro* by Yamashiro *et al.*
Human iPSCs (hiPSCs) were differentiated into human PGCLCs (hPGCLCs) and then matured into oogonia-like cells by culture together with mouse embryonic ovarian somatic cells (top). Although phenotypic and molecular characteristics were consistent with early oogenesis, the oogonia-like cells failed to enter meiosis in the 17-week culture. Whether further development toward mature oocytes can be achieved *in vitro* remains unknown. In humans, oogonial development (bottom panel) occurs during weeks 6 to 10 of gestation, with primordial follicles formed between week 15 and birth. The processes of folliculogenesis, oocyte maturation and ovulation occur after birth and take ~240 days. hPGCs, human primordial germ cells.

Spermatogonial stem cells.
Kubota H, Brinster RL.
Biol Reprod. 2018 Jul 1;99(1):52-74.

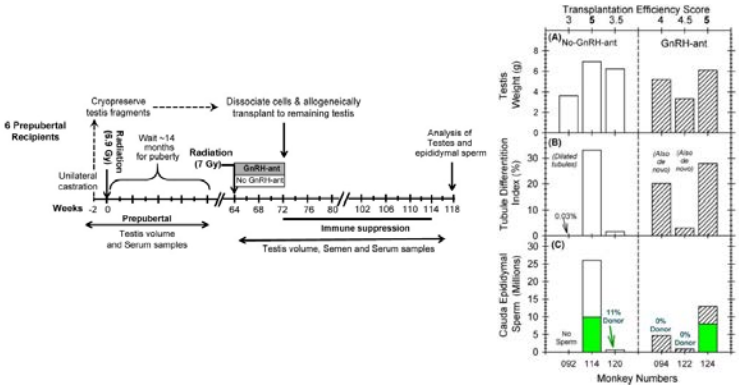


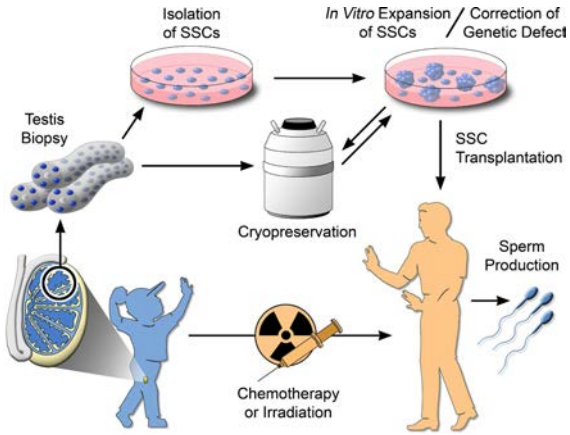
Outline of spermatogonial transplantation method and quantitative assay for SSCs. Single-cell suspension prepared from testes of transgenic mice expressing a reporter gene (e.g., β-galactosidase) by enzymatic digestion is injected into the seminiferous tubules of an infertile recipient mouse. Cells from in vitro culture or cells fractionated by FACS or MACS can be used for a donor cell population. Two months after transplantation, donor-derived spermatogenesis can be detected in the recipient testis as blue colonies. Because each colony of spermatogenesis is developed from a single SSC, the number of colonies represents the number of SSCs in the donor cell suspension. The length of each colony demonstrates the degree of SSC expansion. Modified from [199].

Spermatogonial stem cell transplantation into nonablated mouse recipient testes.
Morimoto H, Ogonuki N, Kanatsu-Shinohara M, Matoba S, Ogura A, Shinohara T.
Stem Cell Reports. 2021 Jul 13;16(7):1832-1844.



Postpubertal spermatogonial stem cell transplantation restores functional sperm production in rhesus monkeys irradiated before and after puberty
Shetty G, Mitchell JM, Lam TNA, et al.
Andrology. 2021 Sep;9(5):1603-1616.





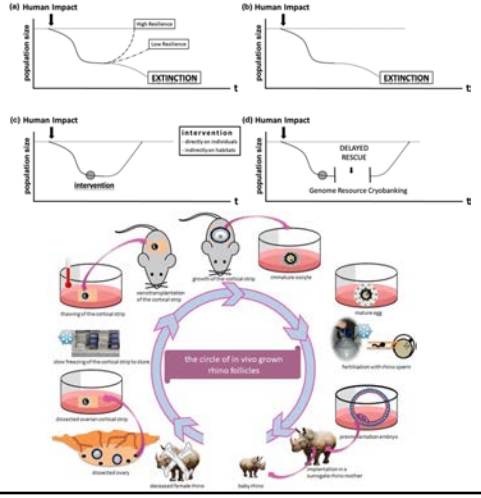
Stem Cell-Derived Human Gametes: The Public Engagement Imperative.
Adashi EY, Cohen IG, Hanna JH, Surani AM, Hayashi K.
Trends Mol Med. 2019 Mar;25(3):165-167.

Abstract

The implications of scientific breakthroughs are rarely faced up to in advance of their realization. Stem cell-derived human gametes, a disruptive technology in waiting, are likely to recapitulate this historic pattern absent active intervention. Herein we call for the conduct of thoughtful ante hoc deliberations on the prospect of stem cell-derived human gametes with an eye toward minimizing potential untoward post hoc regulatory or statutory impositions.

ART and Conservation

The ART of bringing extinction to a freeze - History and future of species conservation, exemplified by rhinos
Hildebrandt TB, Hermes R, Goeritz F, et al.
Theriogenology. 2021 Jul 15;169:76-88.



Reptile assisted reproductive technologies: can ART help conserve 300 million years of evolution by preserving extant reptile biodiversity?

Perry SM, Mitchell MA. Reprod Fertil Dev. 2022 Mar;34(5):385-400.

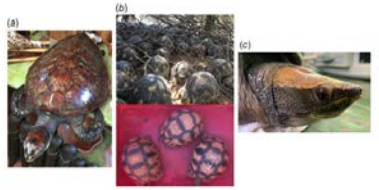


Fig. 1. The global decline in reptiles is a direct result of anthropogenic activities. (a) A green sea turtle (*Chelonia mydas*) poached and for sale in a market. (b) Confiscated radiated tortoises (*Astrochelys radiata*) and ploughshare tortoises (*Astrochelys yniphora*). (c) Central American River turtle (*Dermatemys mawii*) populations were decimated due to overharvesting; however, *in situ* conservation efforts are facilitating a comeback. (Photo credit: Sean Perry.)

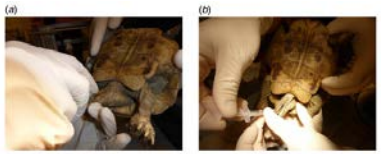
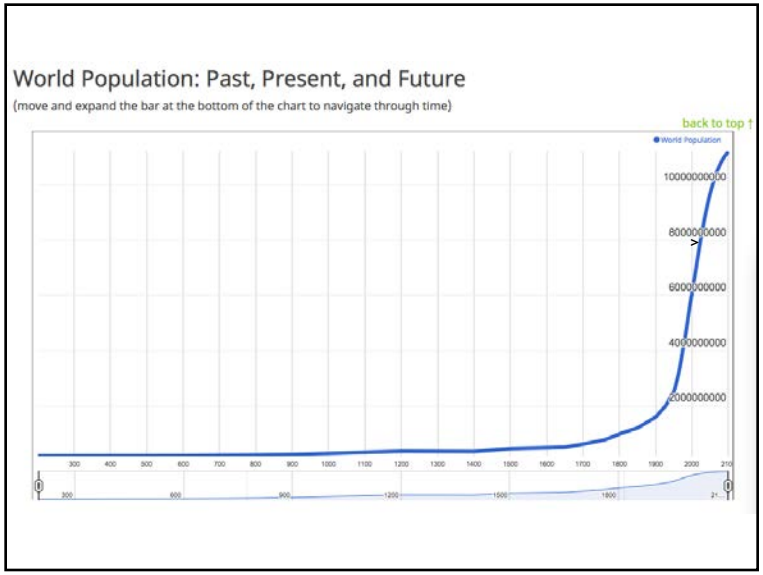
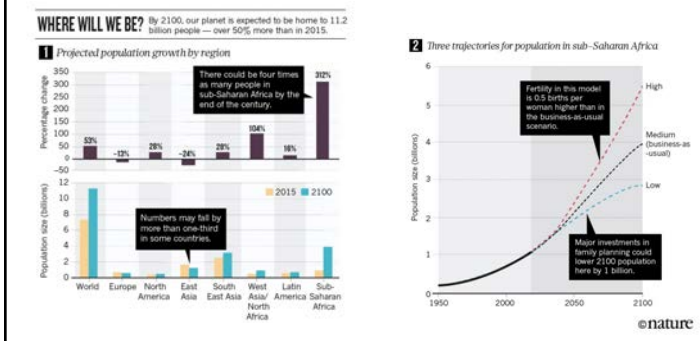


Fig. 2. Electroejaculation in a Hermann's tortoise (*Testudo hermanni*). (a) Electroejaculation probe being inserted into the cloaca for semen collection. (b) Semen collection from the cloaca using a 1 mL syringe following electroejaculation. (Photo credit: Mark Mitchell.)

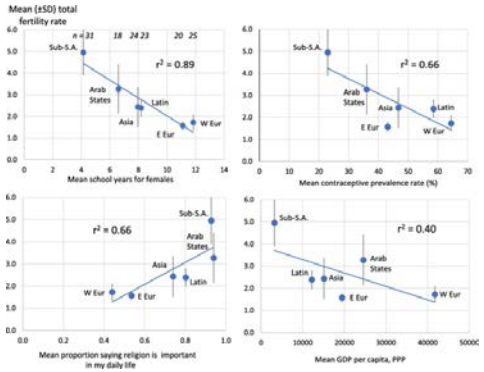
Population and Contraception

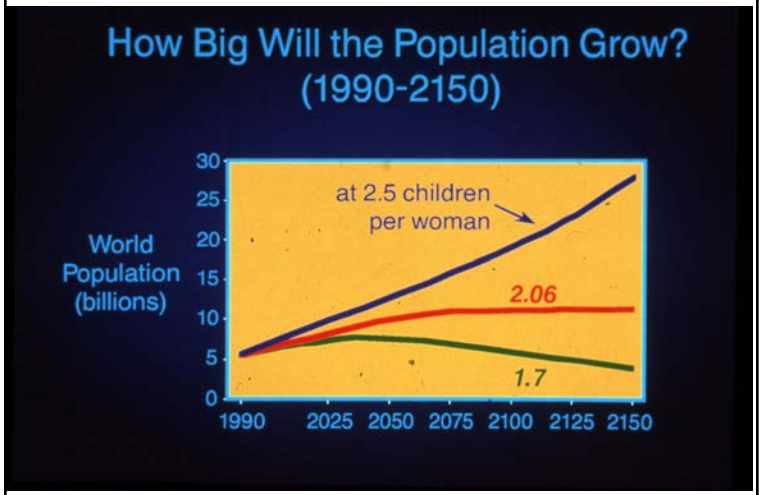


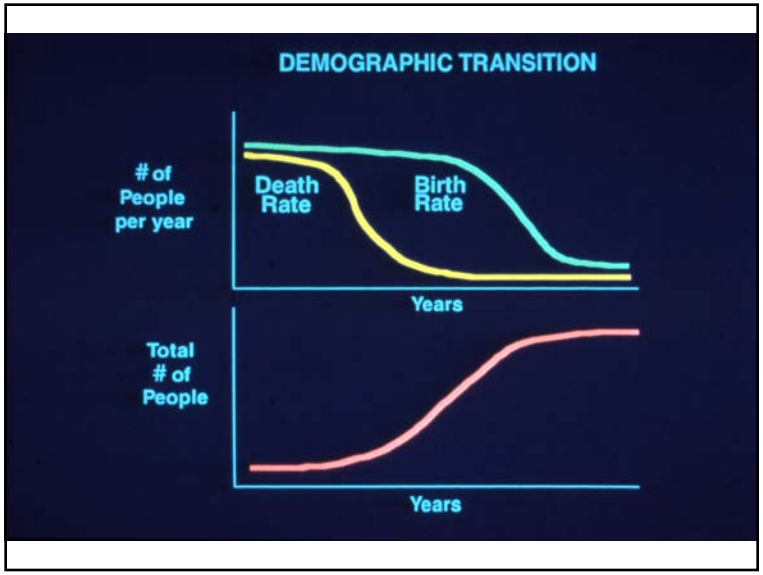
Development: Slow down population growth.
Bongaarts J.
Nature. 2016 Feb 25;530(7591):409-12.

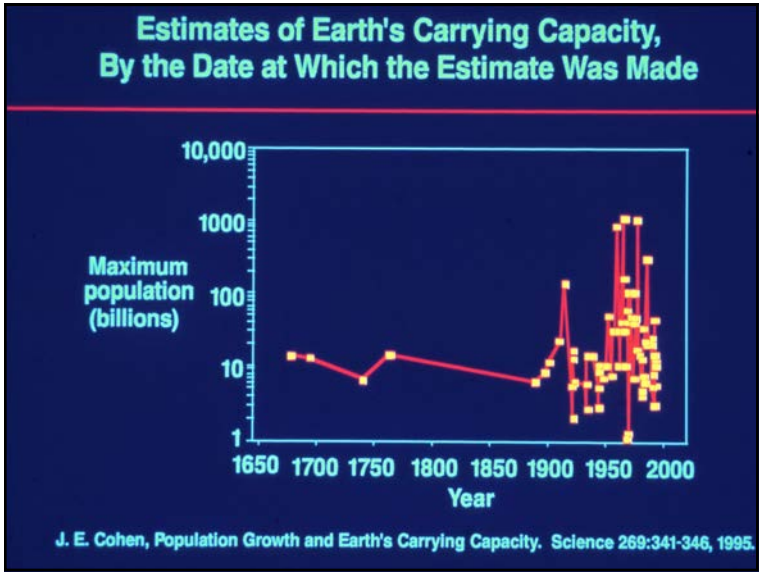


Human fertility in relation to education, economy, religion, contraception, and family planning programs.
Götmark F, Andersson M.
BMC Public Health. 2020 Feb 22;20(1):265.









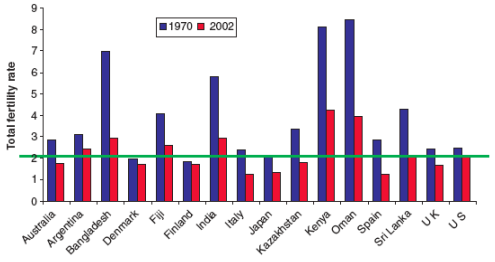
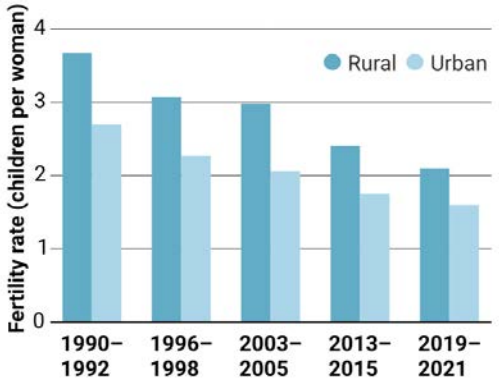


Figure 1 Changes in fertility rates in developing and industrialized countries. The green line is the 2.1 fertility rate, which is necessary to sustain a population at its current level (from World Bank, 2005).

India defuses its population bomb.

Pearce F.

Science. 2021 Dec 17;374(6574):1422-1423.



Steadily shrinking families

Rural women in India tend to have more children than urban women,

TOP 20 LARGEST COUNTRIES BY POPULATION (LIVE)

1	China	1,408,862,597	11	Mexico	126,513,745
2	India	1,295,654,426	12	Philippines	103,271,385
3	U.S.A.	323,697,958	13	Ethiopia	101,041,900
4	Indonesia	258,263,824	14	Vietnam	94,149,075
5	Brazil	205,111,718	15	Egypt	85,863,264
6	Pakistan	190,701,143	16	Germany	82,517,092
7	Nigeria	187,794,356	17	Iran	80,366,737
8	Bangladesh	162,062,611	18	Turkey	77,510,873
9	Russia	141,803,954	19	Congo	72,850,732
10	Japan	126,738,599	20	Thailand	67,611,347

World Population Forecast

Year	Population	Yearly % Change	Yearly Change	Median Age	Fertility Rate	Density (P/Km²)	Urban Pop %	Urban Population
2020	7,758,156,792	1.09 %	81,736,939	31	2.47	60	55.9 %	4,338,014,924
2025	8,141,661,007	0.97 %	76,700,843	32	2.43	63	57.8 %	4,705,773,576
2030	8,500,766,052	0.87 %	71,821,009	33	2.38	65	59.5 %	5,058,158,460
2035	8,838,907,877	0.78 %	67,628,365	34	2.35	68	61 %	5,394,234,712
2040	9,157,233,976	0.71 %	63,665,220	35	2.31	70	62.4 %	5,715,413,029
2045	9,453,891,780	0.64 %	59,331,561	35	2.28	73	63.8 %	6,030,924,065
2050	9,725,147,994	0.57 %	54,251,243	36	2.25	75	65.2 %	6,338,611,492

CONSEQUENCES OF EXCESSIVE POPULATION

Environmental Pollution

Global Warming

Resource Depletion

Starvation

Contraception Throughout the Ages

- **0 Coitus Interruptus:** the most popular contraceptive of all time, is mentioned in the book of Genesis.
- **1850 B.C. The Petrie Papyrus:** an Egyptian medical manual, instructed woman on three pessaries; one of which called for crocodile dung. A pessary is a suppository placed in the vagina to kill sperm, and/or block their path to the cervix; it was possibly the most effective of contraception used in the ancient world.
- **1550 B.C. The Ebers Papyrus:** a compendium of medical practices, contained what was probably the first written description of a contraceptive tampon, made with acacia, honey, dates, and seed wool. (Acacia produces lactic acid, a substance still recognized as a spermicide.)
- **1350 B.C. Egyptian men** wore decorative covers made from linen on their penises.
- **2nd Century The Talmud:** first mentions "spongy substances" used as tampons to prevent sperm from entering the womb.
- **1564 Gabriele Fallopius:** first identified the fallopian tubes, constructed a linen cloth made to fit over the penis - a forerunner of the modern-day condom. Also an early expert on syphilis, Fallopius probably designed the condom as protection against disease, not as a contraceptive.
- **1600s Allegedly,** one Dr. Condon, or Conton, recommended the linen sheath as a means of contraception, and the word condom entered the language.
- **1780s Sponges:** soaked in brandy, became a birth-control fad with the French.
- **1873 The Comstock Law:** made it illegal to send obscene materials through the mail, expressly defined contraceptives and information about birth control as obscene.
- **1880s The Diaghragm** was invented.
- **1880** The process for vulcanizing rubber made it possible to produce mass quantities of cheap condoms. A nickname was born.
- **1909** The modern day IUD: introduced, though versions of the device date back 3,000 years. In the Middle East, legend has it that a pebble was inserted into the uterus of a camel to prevent it from becoming pregnant.
- **1931** The block pessary: "a square block of wood with concave sides that was inserted into the vagina with the expectation that it would prevent semen from entering.

Table 15.1
Modern Birth Control

Strategy	Method
Prevention of gamete formation	
Females	Combined oral contraceptives (COCs)
	Progesterone-only contraceptives (POCs)
	Progesterone-only or combined injectables
	Progesterone analog implants
	GnRH analog/estrogen pills (experimental)
Males	Androgen injectables (in trials)
	Androgen/progesterone injectables (experimental)
	GnRH analog/androgen implants (experimental)
	Anti-FSH vaccine (in trials)
Prevention of fertilization	
	Sterilization
	Tubal ligation in the female
	Vasectomy in the male
	Barriers
	Male condom
	Female condom
	Diaphragm
	Spermicides
	Specific sperm inactivators
	Calcium blockers (experimental)
	Mifepristone (experimental)
	Intrauterine device (IUD)
	Antisperm vaccines (experimental)
	Periodic abstinence (rhythm)
Prevention of implantation	
	Intrauterine device (IUD)
	Postcoital estrogen/progestin
	Mifepristone
	Anti-hCG vaccines (in trials)
Prevention of live birth	
	Medical abortion
	Surgical abortion

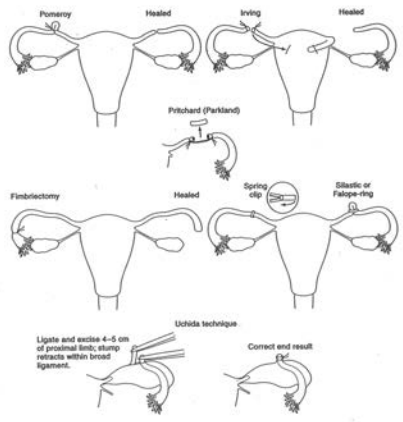


Figure 15.4
Tubal sterilization procedures. The uterine tubes are approached through a small incision in the abdomen. Sterilization is accomplished by ligation, clamping with clips or rings, or electrocoagulation. (Adapted from R. A. Hatcher *et al.*, 1994.)

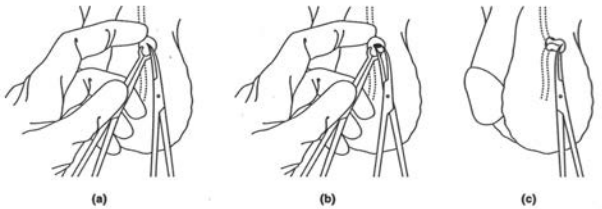


Figure 15.5
"No-scalpel" vasectomy. The vas deferens (dotted line) is grasped by special ring forceps and the skin is pierced by special dissecting forceps (a). The forceps stretch an opening (b), and vas is lifted out and cut or clipped (c). (Adapted from Population Information Program.)

Contraceptive Method	Effect on Reproductive Tract	Effect on Bacterial STDs	Effect on Viral STDs
Diaphragm/Cervical cap/Sponge	Reduces risk of PID; associated with vaginal and urinary infections	Some protection against cervicitis; increases organisms associated with bacterial vaginosis, candidiasis and urinary tract infections	No protection against vaginal infection or external genitalia transmission; prevention of HPV controversial. No protection against HIV
Female condom	Occasional local irritation	In vivo protection against recurrent trichomonal infections suggests possible protection against other STDs	In vitro impermeability to cytomegalovirus, HIV
IUD	Foreign body reaction within the uterus;	Copper IUD: No protection LNG-IUS: associated with decreased upper-genital tract infection	No protection
Latex male condom	Occasional latex allergy	Protection against most pathogens in genital fluids	Less protection against organisms transmitted from external genitalia (HSV and HPV)
Combination oral contraceptive	Increased cervical ectopy; decreased risk of symptomatic PID requiring hospitalization	No protection against bacterial STDs; possible increase in cervical chlamydia	Data on HIV transmission risks conflicting; role regarding risk of HPV infection and cervical dysplasia unclear
DMPA/ Implants	Atrophic endometrium; thickening of cervical mucus	Assume no protection	May promote HIV transmission
Spermicide with nonoxynol-9	Risk of chemical irritation of vaginal epithelium/alteration of the vaginal flora with high doses	equivocal	Data suggests increased HIV transmission risk that is dose and frequency dependent
Tubal ligation	Changes associated with surgery	No protection	No protection
Contraceptive Vaginal rings	Increased Vaginal discharge in some users	No protection	No protection

Table 15.4
Birth Control Methods in the United States, 1995

	% Usage by age				
Method	15–44	15–19	20–24	25–34	35–44
All methods	64.2	29.8	63.5	71.1	72.3
Female sterilization	27.7	0.3	4.0	23.8	45.0
Male sterilization	10.9	0.0	1.1	7.8	19.4
Pill	26.9	43.8	52.1	33.3	8.7
IUD	0.8	0.0	0.3	1.7	1.1
Diaphragm	1.9	0.1	0.6	1.7	2.8
Condom	20.4	36.7	26.4	21.1	14.7
Other	11.4	19.1	15.5	10.6	8.3

The category "Other" in Table 15.4 includes the use of spermicidal jellies, foams, and sponges, all of which act by killing or inactivating sperm. Also included in this category is the rhythm method, in which intercourse is avoided during the days before and after ovulation. (Reference: National Center for Health Statistics. Health, United States, 1998, with Socioeconomic Status and Health Chartbook.)

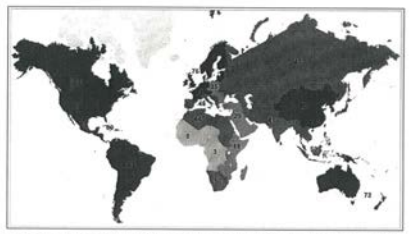


FIG. 1. Contraceptive prevalence by regions of the world. Data are percentages of married women currently using modern methods of contraception (male and female sterilization, intrauterine contraceptive device, the pill, injectables, hormonal implants, condoms, and female barrier methods) and vary from 8% in Western Africa to 80% in Eastern Asia, with narrower variation in the more developed regions. Modern methods account for approximately 80% of contraceptive usage in less developed areas but for 70% in more developed areas, with traditional methods, e.g., withdrawal and calendar rhythm methods, which require male involvement, accounting for 20% in developed regions compared with 8% in less developed areas. Data are from the United Nations Population Fund (323).

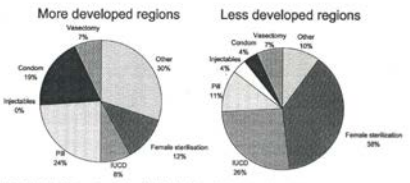


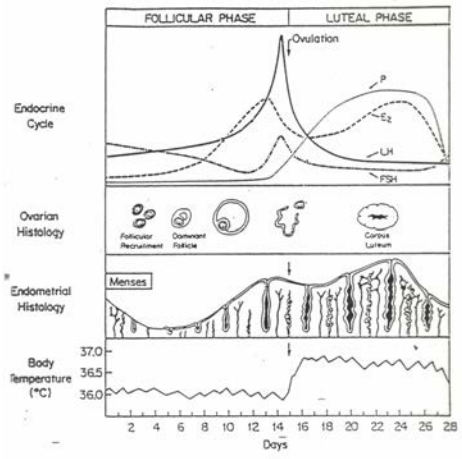
FIG. 2. Distribution of contraceptive usage by method in developed vs. less developed regions of the world. Seventy percent of users in the more developed areas rely on short-acting, reversible methods (condoms, pills, traditional methods), whereas in the less-developed areas, 70% use longer-acting, clinic-based methods (injectables, sterilization, intrauterine contraceptive devices). Data are from the United Nations Population Fund (319).

Table 2. **Contraception Efficacy Rates***

Method	Unplanned Pregnancy in the First Year of Use, %	
	Typical Use	*Perfect Use*
No method	85	85
Withdrawal	20	4
Cap		
Parous women	32	26
Nulliparous women	16	9
Sponge		
Parous women	27	20
Nulliparous women	14	9
Diaphragm	17	16
Condom		
Female	21	5
Male	13	2
Combined hormonal contraceptive pill	7	0.3
Progesterone-only pill	7	1
Transdermal patch	7	0.3
Intravaginal ring	7	0.3
Depo-Provera	4	0.2
Subdermal implant	0.1	0.1
Copper IUD	0.8	0.6
LNG-IUD	0.1	0.1
Female sterilization (tubal occlusion)	0.5	0.5
Male sterilization	0.15	0.1

IUD intrauterine device; LNG = levonorgestrel.
**Percentage of women with an unintended pregnancy during first year of typical use and perfect use (17).*

Female contraceptive



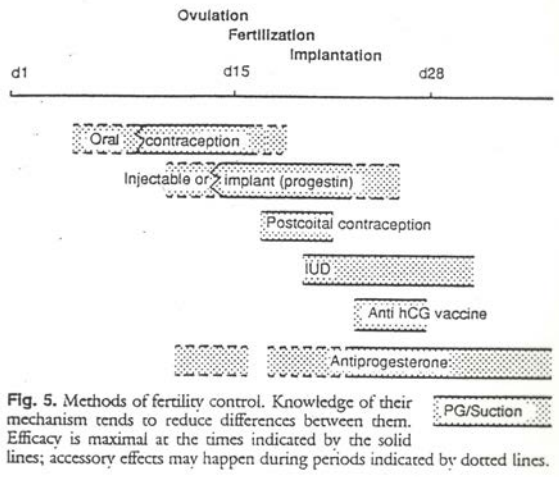


Fig. 5. Methods of fertility control. Knowledge of their mechanism tends to reduce differences between them. Efficacy is maximal at the times indicated by the solid lines; accessory effects may happen during periods indicated by dotted lines.

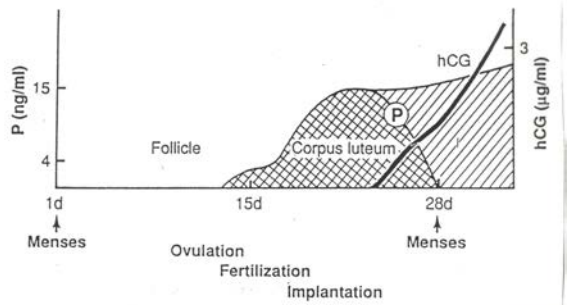


Fig. 1. Plasma concentrations of progesterone (P) (light line) during nonfertile (cross-hatched) and fertile (diagonal lines) cycles, and of hCG (heavy line) in case of fertilization and implantation; d, day.

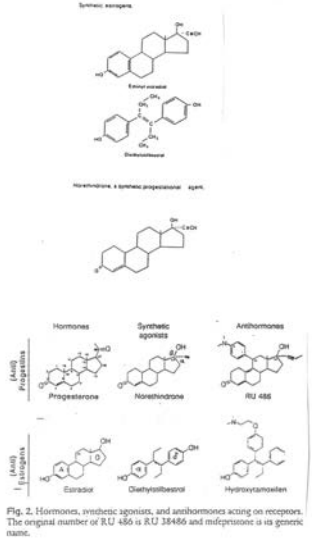


Fig. 2. Hormones, synthetic agonists, and antihormones acting on receptors. The original number of RU 486 is RU 38486 and mifepristone is its generic name.

Table 1. **Pharmacologic and Barrier Methods of Contraception**

Type	*Examples*	*Dosing Frequency*	*Comments*
Combined (ethinyl estradiol and a progestin)	Oral contraceptive pills (COCs, "the pill")	Taken daily by mouth	Efficacy for all short-acting methods depends on user compliance
	Transdermal patch (Xulane)	Single patch changed weekly for 3 wk, then off for 1 wk (for withdrawal bleeding)	
	Vaginal ring (Nuvaring)	Inserted in the vagina for 3 wk, then removed for 1 wk (for withdrawal bleeding); new ring every mo	
Progesterone only	Oral contraceptive pills (POPs, "mini pill")	Taken daily by mouth with no "off" wk	Efficacy depends on user compliance
	Injectable (medroxyprogesterone [Depo-Provera])	Intramuscular or subcutaneous injection every 3 mo	Intramuscular: Must be given by a health care provider; self-administered subcutaneous injection is off-label
LARC methods	Subdermal implant (Nexplanon)	Placed subdermally in upper arm, approved for 3 years, effective to 4	Must be placed by a trained health care provider; dosing frequency reflects the longest amount of time for which the method can be used; the device can be removed earlier
	LNG-IUD [Liletta, Mirena, Kyleena, Skyla])	Inserted in uterus, approved for 3-5 y; Liletta and Mirena are effective up to 7 y	
	Copper IUD (Paragard)	Inserted in uterus, approved for 10 y, effective for 12 y	
Barrier	Condoms (male and female)	Every intercourse	The only methods of contraception that also protect against sexually transmitted diseases; available over the counter
	Vaginal sponges	Every intercourse	Available over-the-counter
	Diaphragm, cervical cap	Every intercourse	Must be fitted by a physician

COC = combined oral contraceptive; IUD = intrauterine device; LARC = long-acting reversible contraception; LNG = levonorgestrel; POP = progesterone-only pill.

Fifty years of "the pill": risk reduction and discovery of benefits beyond contraception, reflections, and forecast.

Chadwick KD, Burkman RT, Tornesi BM, Mahadevan B.

Toxicol Sci. 2012 Jan;125(1):2-9.

Abstract

Widely regarded as a revolutionary drug in its early years, "the pill" may be considered the first designer or lifestyle drug. Approximately 85% of women in the United States will use an oral contraceptive (OC) for an average of 5 years. Since the introduction of OCs in the 1960s, both health benefits and safety concerns have been attributed to their use. Widespread use of OC formulations by women throughout their reproductive life cycle gave rise to concerns about the effects of OCs on risk factors for cardiovascular disorders and cancer. In most instances, the noncontraceptive benefits of OCs outweigh the potential risks. As with many first in class drugs, lessons can be learned from its development and use. Indeed, "the pill" played a significant role in reshaping the regulatory process for new drugs during the second half of the 20th century. The birth control pill celebrates its 50th birthday this year, as women and men celebrate five decades of this revolutionary method of family planning. Recent scientific and technological advances in genomics, proteomics, new materials, and new drug delivery systems, along with a new understanding of reproductive biology, offer the promise of new, safe, and effective forms of contraception. In addition to the history of OC therapeutic advances and unintended side effects, the noncontraceptive health benefits that women experience beyond pregnancy prevention are discussed. This article summarizes a symposium presented at the 50th Anniversary of the Society of Toxicology National Meeting, held from 6 to 10 March 2011 in Washington, DC.

Table 3 Advantages of oral contraceptives (29)

Reduction of ovarian and endometrial cancer risk;

- Reversibility and quick return to fertility;
- Reduced risk of benign ovarian tumors and ovarian cysts
- Reduced risk of colorectal cancer
- Reduced dysfunctional uterine bleeding
- Decreased perimenopausal vasomotor symptoms
- Decreased benign breast disease
- Favorable bone mineral density profile
- Decrease in menstrual flow and menorrhagia
- Decrease in primary dysmenorrhea
- Decreased risk of iron deficiency anemia
- Improvement in hirsutism and acne
- Decreased risk of premenstrual syndrome (PMS)/premenstrual dysphoric disorder (PMDD)

Exogenous sex hormones, menstrual and reproductive history, and risk of non-melanoma skin cancer among women: a systematic literature review and meta-analysis
Caini S, De Angelis SP, Corso F, et al.
Sci Rep. 2021 Apr 19;11(1):8524.

Abstract

Non-melanoma skin cancers (NMSC) are more frequent among men, but women (especially those aged < 40 years) have experienced steeper growth in their incidence rates in recent years. Hormonal factors were hypothesized to be playing a role in modulating NMSC risk, but the studies published to date provided conflicting results. We systematically reviewed and meta-analysed the studies focusing on the association between hormone-related characteristics (use of exogenous sex hormones, and aspects of menstrual and reproductive history) and the risk of NMSC among women. We included observational and experimental studies published in PubMed and EMBASE until February 2020. We calculated summary relative risk (SRR) and 95% confidence intervals (CI) by applying random effects models with maximum likelihood estimation, and used the I^2 statistics to quantify the degree of heterogeneity of risk estimates across studies. Eleven independent studies encompassing a total of over 30,000 NMSC cases were included in quantitative analyses. No evidence of an increased NMSC risk emerged among ever vs. never users of oral contraceptives (SRR 1.13, 95% CI 0.88-1.45) or hormones for menopause (SRR 1.09, 95% CI 0.87-1.37). Likewise, age at menarche or at menopause and parity were not associated with NMSC risk. Heterogeneity across studies was low, and pooled results were comparable between NMSC subtypes. We found no evidence that hormonal factors play a role in the pathogenesis of NMSC among women.

Combined oral contraceptive use in rheumatoid arthritis for the purpose of pregnancy prevention and additional benefits: A narrative review.
Lopane CM, Comstock B, Nagel AK, Gandhi MA.
J Obstet Gynaecol Res. 2022 Feb;48(2):306-312.

TABLE 1 Most common disease-modifying antirheumatic drugs used in patients with rheumatoid arthritis[9,10]

Brand	Generic	Drug class	Typical dose for RA
Rheumatrex®, Trexall®	Methotrexate	Antimetabolite	20 mg per week orally or subcutaneously
Plaquenil®	Hydroxychloroquine	Antimalarial	200–400 mg daily orally
Arava®	Leflunomide	Pyrimidine synthesis inhibitor	20 mg daily orally following 3 days of 100 mg daily orally
Azulfidine®	Sulfasalazine	Sulfonamides, 5-ASA	2–3 g daily split into two doses
Orencia®	Abatacept	T-cell costimulatory blocking agent	<60 kg: 500 mg 60–100 kg: 750 mg > 100 kg: 1 g All given IV infusion on Days 1, 14, and 28 then every 4 weeks
Enbrel®	Etanercept	Tumor necrosis factor (TNF) inhibitor	50 mg once weekly subcutaneously
Actemra®	Tocilizumab	IL-6 inhibitor	4 mg/kg IV drip then increased to 8 mg/kg IV drip every 4 weeks
Remicade®	Infliximab	Tumor necrosis factor (TNF) inhibitor	3–10 mg/kg on Days 1, 14, and 42 then every 8 weeks IV infusion
Humira®	Adalimumab	Tumor necrosis factor (TNF) inhibitor	40 mg subcutaneously every 2 weeks

Table 15.12
Modern Abortion Methods

Method	Time in Gestation
Medical abortion	
Prostaglandins alone	Through week 3
Mifepristone/misoprostol	Through weeks 7–8
Methotrexate/misoprostol	Through week 9
Mifepristone/gemeprost	Through week 19
Surgical abortion	
Vacuum aspiration	Through weeks 7–8
Dilation and evacuation	Second trimester
Instillation	Second trimester
Hysterotomy	Third trimester

Misoprostol and gemeprost are prostaglandin analogs. Instillation refers to the injection of toxic compounds into the amniotic sac.

Table 3. **Emergency Contraception Methods***

Method	Dose	Comments
Copper IUD		Reduces risk for pregnancy by 99%; is the most effective emergency contraception
ella	Ulipristal acetate selective progesterone receptor modulator (30 mg pill, given once)	Maintains efficacy though days 1 through 5; available by prescription only; most effective of the dedicated oral regimens
LNG single-dose (Plan B, Plan B One-Step, generics)	1.5 mg LNG taken within 5 days of unprotected intercourse	Diminishing efficacy from day 1 to day 5; may be less effective in women with BMI >26 kg/m^2; least effective of the dedicated oral regimens (risk for pregnancy reduced by 60%–90%)
"Yuzpe" regimen	Combines oral contraceptive pills containing ethinyl estradiol (200 mcg total) and either norgestrel (2 mg) or LNG (1 mg), given once within 72 hr of unprotected intercourse	Reduces risk for unplanned pregnancy by 74%; associated with nausea and vomiting

IUD = intrauterine device; LNG = levonorgestrel.
**From references 25 to 27.*

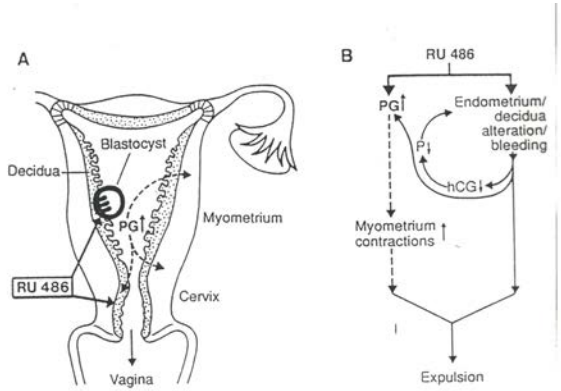


Fig. 4. Effects of RU 486 administered in early pregnancy.

Usage patterns and attitudes towards emergency contraception: the International Emergency Contraception Research Initiative.
Eur J Contracept Reprod Health Care. (2016) 21(4):310-7.
Krassovics M, Virágh G.

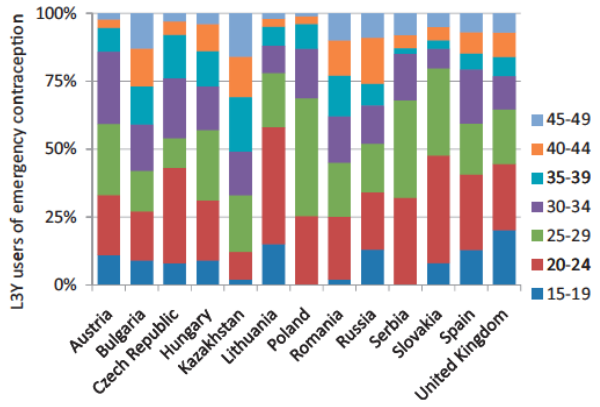


TABLE 23–1. MECHANISM OF ACTION OF ESTROGEN/PROGESTIN CONTRACEPTIVES

Inhibition of ovulation by suppression of FSH and LH
Alteration of cervical mucus to inhibit sperm transport
Interference with ovum transport
Inhibition of implantation by suppression of normal endometrial development

Characteristics and metabolic effects of estrogen and progestins contained in oral contraceptive pills. Sitruk-Ware R, Nath A.

Best Pract Res Clin Endocrinol Metab. 2013 Feb;27(1):13-24.

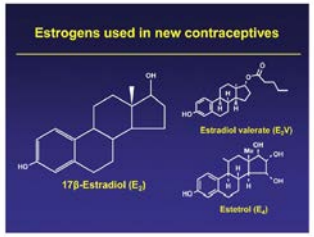


Fig. 1. Structures of natural estrogens used in new contraceptives.

Table 1
Classification of the progestins used in contraception according to chemical structure.

Progestin group	Molecules
Structurally related to testosterone	
Estranes and Gonanes	*Estranes*: structurally related to testosterone, norethisterone, norethisterone acetate, norethynodrel *Estranes (non-ethinylated)* e.g. dienogest
13-ethyl gonanes	Levonorgestrel Desogestrel (etonogestrel), gestodene, norgestimate (norelgestromine).
Structurally related to progesterone	
Pregnanes and norpregnanes	*Pregnanes*: structurally related to 17-hydroxyprogesterone e.g. medroxyprogesterone acetate, cyproterone acetate, chlormadinone acetate
19-norprogesterones (*19-norpregnanes*)	(Non acetylated): trimegestone, nestorone (Acetylated) nomegestrol acetate
Spironolactone derivative	Drospirenone

Values in italics indicate sub-classes of progestins.

Table 1. Historical antecedents for the development of progestin implants

1964	Diffusion of biologically active substances through Silastic membranes [1]
1965	Oral contraception by continuous low dose progestin [2]
1966	Diffusion of steroids through Silastic membranes [3,4]
1967	First clinical trial with progestin-releasing Silastic capsules [5]

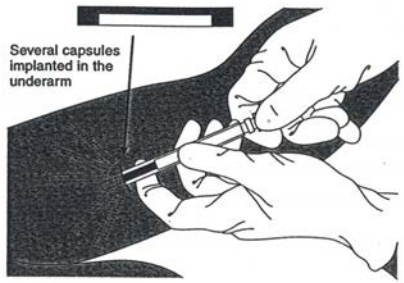


Figure 15.3
Schematic illustration of subdermal capsules and their implantation in the underarm.

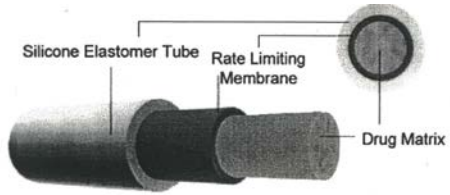


Fig. 4. Design of the Nestorone® single-rod implant being developed by the Population Council. Nestorone microcrystals are embedded in a silicone elastomer matrix (Courtesy of Dr Alfred Moo-Young).

Long-acting injectable hormonal dosage forms for contraception.
Wu L, Janagam DR, Mandrell TD, Johnson JR, Lowe TL.
Pharm Res. 2015 Jul;32(7):2180-91.

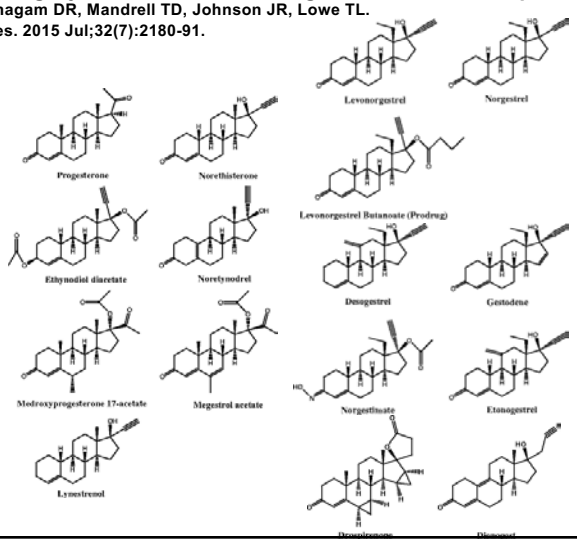


Table 2. Progestin implants

Progestin	Tradename	Units	Duration of action
Levonorgestrel	Norplant®	Six capsules	5 years
Levonorgestrel	Jadelle®	Two rods	5 years
Etonogestrel	Implanon®	Single rod	3 years
Nestorone	Elcometrine®	Single capsule	6 months
Nestorone		Single rod	2 years
Nomegestrol Acetate	Uniplant® or Surplant®	Single rod	1 year

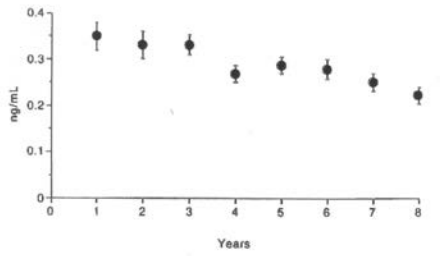


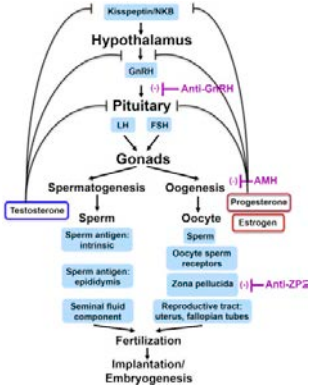
Fig. 2. Levonorgestrel serum levels in women using the same set of Norplant® for 8 years. Each dot and bar represent the average ± SE of over 20 subjects sampled in the corresponding year.

Table 5. Typical first-year pregnancy and continuation rates of various methods[legend]

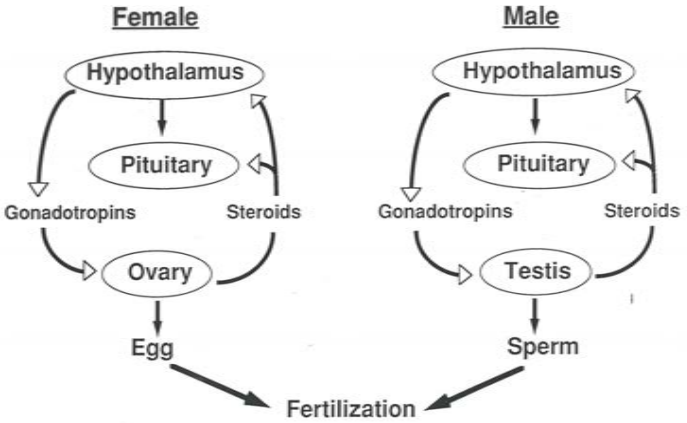
Method	Accidental pregnancy %	Continuation %
Condom	12.0	63
Pill	3.0	72
Copper T380	0.8	78
Norplant®	< 0.1	85
Female sterilization	0.4	99

[legend] From Ref. [8].

Vectored gene delivery for lifetime animal contraception: Overview and hurdles to implementation.
Theriogenology. 2018 May;112:63-74.
Hay BA, Li J, Guo M.



Some potential targets of interest for vectored contraception. Some, but not all, points at which antibodies, ligands, or small RNAs could interfere with fertility are indicated. Proteins or structures of particular interest are indicated in the blue boxes. Receptors for GnRH, FSH, and LH, located on target tissues are also of interest, but are not indicated. Negative feedback pathways mediated by steroid hormones are complex. They are indicated in simplified form by the black lines with a bar on the end. Antibodies and molecules targeted by Li et al. [9] and Kano et al. [10] are indicated in purple. Many targets of interest, including molecules required for embryo implantation and development, are untested. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

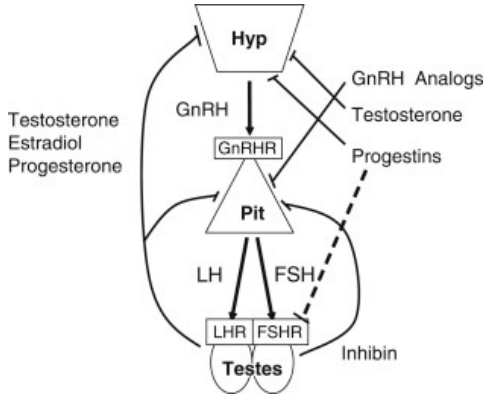


Male Contraceptive

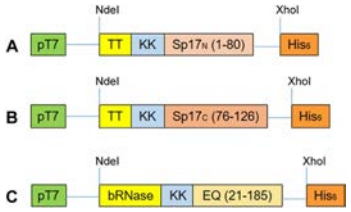
Deficiencies in Present Male Methods

- Condom
  - Poor compliance
  - Poor efficacy
  - Coitally related
- Vasectomy
  - Non-reversible
- Interrupted coitus
  - High failure rate

A hormonal contraceptive for men: how close are we?
Huhtaniemi I.
Prog Brain Res. 2010;181:273-88.



Novel Sperm and Gonadotropin-releasing Hormone-based Recombinant Fusion Protein: Achievement of 100% Contraceptive Efficacy by Co-immunization of Male and Female Mice.
Mol Reprod Dev. 2016 Dec;83(12):1048-1059.
Minhas V, Shrestha A, Wadhwa N, Singh R, Gupta SK.



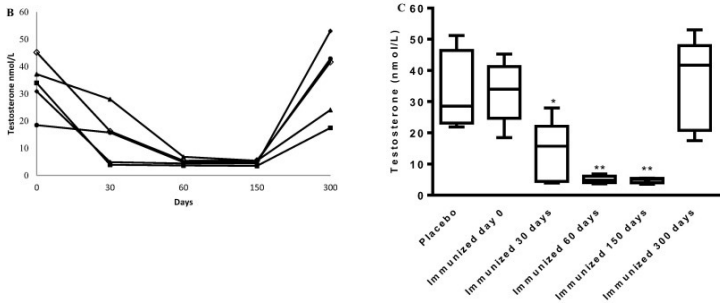
Constructs used for expression of the recombinant proteins. A: N-terminal fragment of mouse Sp17 (Sp17N), amino acid residues 1–80, linked to the T non-B cell epitope of TT (amino acid residues 830–844) by a di-lysine linker (KK) at the N-terminus. B: C-terminal fragment of mouse Sp17 (Sp17c), amino acid residues 76–126, linked to TT by KK at the N-terminus. C: Mouse equatorin (EQ), amino acid residues 21–185, linked to the T non-B cell epitope of bovine RNase (bRNase; amino acid residues 94–104) by KK at the N-terminus. The respective restriction sites used to clone the inserts into the pET22b(+) expression vector are shown. pT7, T7 promoter; His6, histidine tag.

TABLE 3. Immunogenicity and Contraceptive Efficacy of Recombinant $Sp17_C$-$GnRH_2$ in FVB/J Female Mice

Immunogen	Antigens used for ELISA	Antibody titers, AU × 10^3 Day 0	Day 35	Day 56	Percentage of animals that failed to conceive	Pups per mated female	*P*-value of Ig titers in pregnant versus non-pregnant mice (Day 56)
Alum only (n = 10)	$Sp17_C$-$GnRH_2$	<0.05	<0.05	<0.05	0	7.70 ± 0.30	NA
$Sp17_C$-$GnRH_2$ (n = 10)	$Sp17_C$-$GnRH_2$	<0.05	64.60 ± 12.75	169.20 ± 35.11	80	1.70 ± 1.13	0.05
	TT-KK-$Sp17_C$	<0.05	14.00 ± 1.04	59.20 ± 4.25			0.01
	GnRH-BSA	<0.05	4.28 ± 0.83	15.70 ± 1.45			0.04

Data presented as mean ± standard error. NA, not applicable.

A dual kisspeptin-GnRH immunogen for reproductive immunosterilization
Junco JA, Fuentes F, Millar RP.
Vaccine. 2021 Oct 15;39(43):6437-6448.



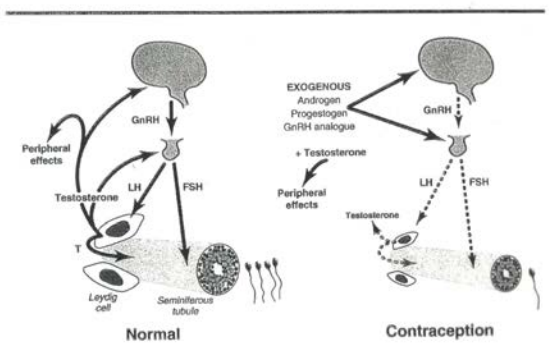
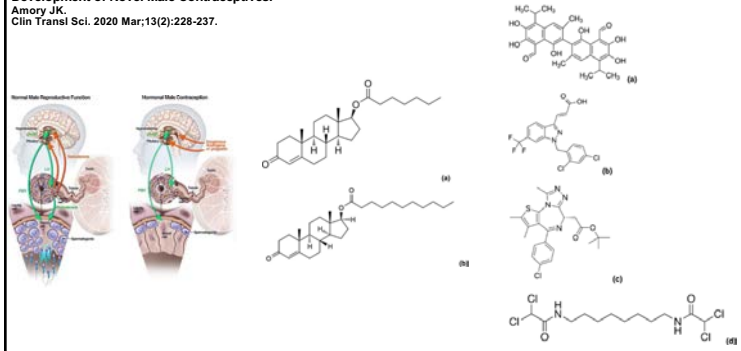


Fig. 1 Representation of the basic principles of hormonal male contraception. The left-hand diagram reflects the physiological relationships between the hypothalamus, anterior pituitary and testis resulting in the production of spermatozoa and of testosterone with both intratesticular and peripheral effects. During exogenous administration of androgen, progestogen or GnRH analogue, gonadotrophic support of the testis is withdrawn resulting in loss of sperm and testosterone production, the latter requiring the administration of testosterone to prevent hypogonadal symptoms.

Development of Novel Male Contraceptives.
Amory JK.
Clin Transl Sci. 2020 Mar;13(2):228-237.



Nonhormonal male contraceptives (**a**) Gossypol, (**b**) H2-gamendazole, (**c**) JQ-1, (**d**) Win 18,446.

The use of testosterone as a male contraceptive.
Baillieres Clin Endocrinol Metab. 1998 Oct;12(3):471-84.
Amory JK, Bremner W.

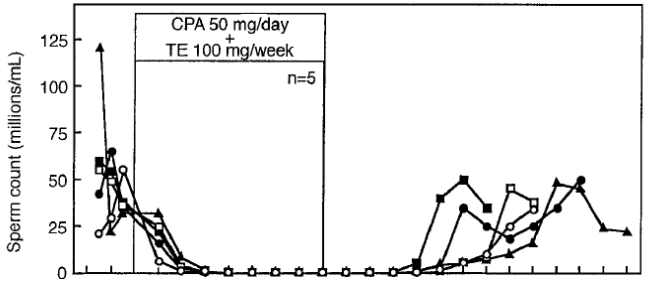


Figure 1. Sperm concentrations in individual subjects during the control period, throughout 16 weeks of hormone administration and during 26 weeks of the recovery phase. Reproduced from Meriggiola et al (1996, *Journal of Clinical Metabolism* **81:** 3018–3023) with permission of the Endocrine Society.

	Number enrolled	Sperm concentration* (million per mL)	Failed to suppress†	Median time to enter efficacy (months)	Entered efficacy	Maximum treatment duration (months)	Pregnancies during efficacy	Exposure (person-years)	Failure rate per 100 couple-years
Testosterone enanthate‡	271	0	68 (25%)	<6	157	18	1 (1%)	123·8	0·8 (0·02–4·5)
Testosterone undecanoate§	1045	1	43 (5%)	3·6	855	30	9 (1%)	1554·1	1·1 (0·4–1·8)
Testosterone implant¶	55	1	2 (4%)	1·8	51	18	0	35·5	0 (0·0–8·0)
Testosterone enanthate‖	399	3‖	Not reported‖	2·2	349	18	11 (3%)	279·9	1·4 (0·4–3·7)
Testosterone undecanoate§	308	3	9 (3%)	2–3	296	12	1 (<1%)	143	2·3 (0·5–6·2)
Total (0, 1, and 3 million per mL)	2078	0, 1, 3	122 (7%)	–	1708	12–30	22 (1%)	2136·3	1·0 (0·7–1·6)**
Total (0, 1 million per mL)	1371	0, 1	113 (8%)	–	1063	18–30	10 (1%)	1713·4	0·6 (0·3–1·1)**
Total (1 million per mL)	1100	1	45 (4%)	–	906	18–30	9 (1%)	1589·6	0·6 (0·3–1·1)**

We did statistical analyses with SAS version 9.3 genmod procedure to calculate aggregate point and asymptotic 95% confidence limits for Poisson events. *Sperm concentration needed to enter efficacy. †Failed to suppress to sperm concentration needed to enter efficacy by 6 months. ‡Testosterone enanthate 200 mg/week. §Testosterone undecanoate 500 mg/month (with 1000 mg loading dose). ¶Implant 800 mg every 4 months with depot medroxyprogesterone acetate (DMPA) 300 mg every 3 months. ‖Original threshold was 5 million per mL, but data shown here are for those who suppressed to no more than 3 million per mL. **Studies pooled and 95% Poisson confidence limits calculated with SAS version 9.3.

Table 2: Contraceptive efficacy of male hormonal contraceptive regimens

Male hormonal contraceptive - are we there yet?
Page ST, Amory JK.
Nat Rev Endocrinol. 2018 Dec;14(12):685-686.

Table 1 | **Efficacy trials of male hormonal contraceptives**

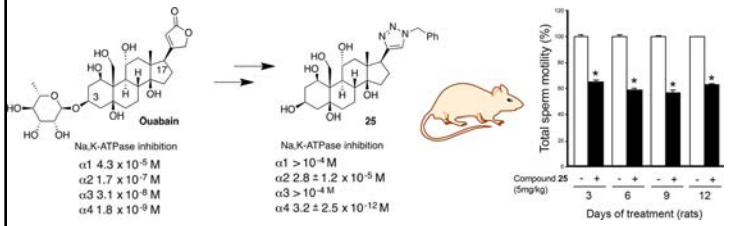
Study	Number of couples enrolled	Number of couples entering efficacy phase[a]	Number of men failing to suppress sperm production[b] (%)	Number of unintended pregnancies (%)	Total number of failures[c] (%)	Overall contraceptive efficacy(%) (95% CI)	Refs
WHO	399	357	8 (2.0)	5 (1.4)	19 (5.3)	94.7 (92–97)	1
Gu et al.	305	296	9 (2.9)	1 (0.1)	16 (5.2)	94.8 (91–97)	2
Gu et al.	1,045	855	43 (4.8)	9 (1.1)	52 (6.1)	93.9 (92–96)	3
Turner et al.	55	53	2 (3.6)	0 (0)	2 (3.6)	96.4 (86–100)	4
Behre et al.	320	266	11 (4.1)	4 (1.5)	20 (7.5)	92.5 (89–95)	5
Total	2,124	1,827	73 (4.0)	19 (1.0)	109 (6.0)	94.1 (93–95)	1–5

CI, confidence interval. [a]For clarity, men or couples who dropped out for reasons other than failure to suppress, pregnancies or sperm rebound are omitted from the efficacy evaluable population. [b]Predefined as sperm concentration of <1–5 million/ml depending on the study. [c]Defined as the sum of the number of pregnancies, failure to suppress sufficiently to be included in the efficacy phase or early discontinuation owing to sperm rebound.

	Target	Mechanism	Adverse events	Tested in human beings
Testis				
Gossypol	Unknown (presumed seminiferous tubules)	Inhibition of spermatogenesis and sperm motility through oxidative stress	Irreversible infertility in up to 20%; dose-dependent hypokalaemia	Yes
Triptolide	Unknown (presumed seminiferous tubules)	Unknown	Irreversible infertility	Yes
Indenopyridine enantiomers and derivatives (CDB 4022, I-CDB 4022, I-RTI-4582-073)	Unknown (presumed Sertoli cell)	Unknown	Species-dependent irreversible infertility	No
Lonidamine derivatives (H2-gamendazole and adjudin)	Apical ectoplasmic specialisation (Sertoli–germ-cell junction)	Disruption of Sertoli–germ-cell junction preventing sperm maturation	Liver inflammation, muscle atrophy, infertility	No
JQ1	Bromodomain testes-specific protein	Impairs chromatin remodelling during spermatogenesis	None in mice	No
BMS 189453	Antagonist of retinoic acid receptors α, β, γ	Blocks spermatogonial differentiation	None noted at low doses	No
BMS 189532 and 195614	Retinoic acid receptor α antagonist	Blocks spermatogonial differentiation	None noted	No
WIN 18,446 (BDAD)	Aldehyde dehydrogenase 1A2 (ALDH1A2) inhibitor preventing conversion of retinaldehyde (vitamin A) to retinoic acid	Blocks spermatogonial differentiation through suppression of STRA8 expression	Impairs liver aldehyde dehydrogenase resulting in disulfiram like reaction with alcohol	Yes
Epididymis				
None	HE6 (G-protein-coupled receptor)	Defect of reabsorption of testicular fluids in epididymal ductules	Unknown	No
None	CRISP-1 glycoprotein (secreted by epididymal epithelium)	Suppresses sperm capacitation and inhibits sperm–egg fusion in rats and mammals	Unknown	No
Sperm motility and sperm–egg fusion				
None	EPPIN (epididymal protease inhibitor)	Binding of semen coagulation protein (semenogelin 1), which impairs sperm motility	Unpredictable irreversible infertility; variable immune response	No
Miglustat (N-butyldeoxynojirimycin)	Glycosphospholipid biosynthesis inhibitor (mice only)	Impairs sperm motility	No effect on sperm in human beings; gastrointestinal symptoms and weight loss	Yes
HC-056456	CatSpers: calcium-permeable ion channels	Mutation or defect of CatSper genes prevents sperm hyperactivation; reduced sperm motility	No other phenotype reported in knockout mice	No
S-3-chlorolactaldehyde	Glyceraldehyde-3-phosphate dehydrogenase-S (GAPDHS; sperm-specific glycolysis; GAPD2 human-specific homologue of GAPDS)	Inhibits sperm-specific glycolysis	Many	No

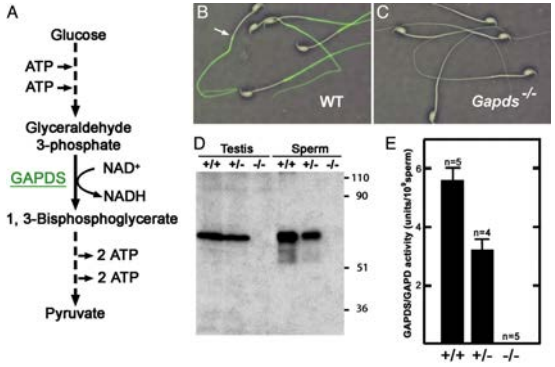
Table 1: **Non-hormonal male contraceptive drugs or potential drug targets**

Design, Synthesis, and in Vitro and in Vivo Evaluation of Ouabain Analogues as Potent and Selective Na,K-ATPase α4 Isoform Inhibitors for Male Contraception.
J Med Chem. 2018 Mar 8;61(5):1800-1820.
Syeda SS, Sánchez G, Hong KH, Hawkinson JE, Georg GI, Blanco G.



Na,K-ATPase α4 is a testis-specific plasma membrane Na^+ and K^+ transporter expressed in sperm flagellum. Deletion of Na,K-ATPase α4 in male mice results in complete infertility, making it an attractive target for male contraception. Na,K-ATPase α4 is characterized by a high affinity for the cardiac glycoside ouabain. With the goal of discovering selective inhibitors of the Na,K-ATPase α4 and of sperm function, ouabain derivatives were modified at the glycone (C3) and the lactone (C17) domains. Ouabagenin analogue **25**, carrying a benzyltriazole moiety at C17, is a picomolar inhibitor of Na,K-ATPase α4, with an outstanding α4 isoform selectivity profile. Moreover, compound **25** decreased sperm motility in vitro and in vivo and affected sperm membrane potential, intracellular Ca^{2+}, pH, and hypermotility. These results proved that the new ouabagenin triazole analogue is an effective and selective inhibitor of Na,K-ATPase α4 and sperm function.

Fig. 1. Targeted disruption of Gapds



Miki, Kiyoshi et al. (2004) Proc. Natl. Acad. Sci. USA 101, 16501-16506

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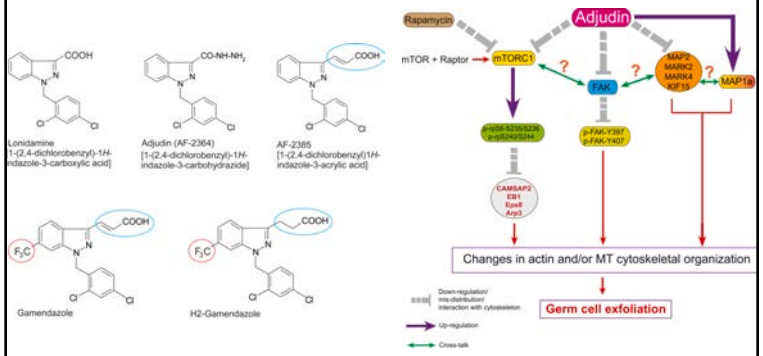
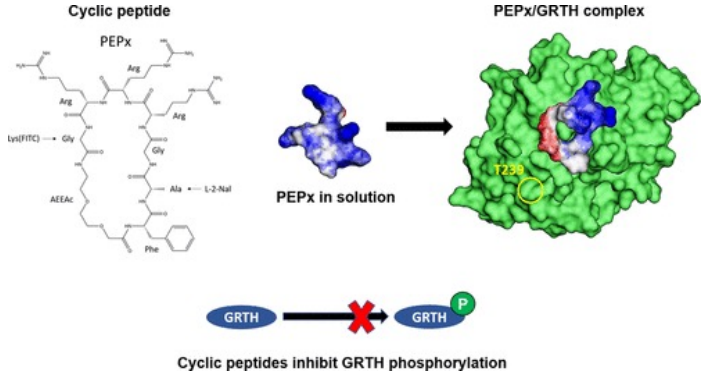
PNAS

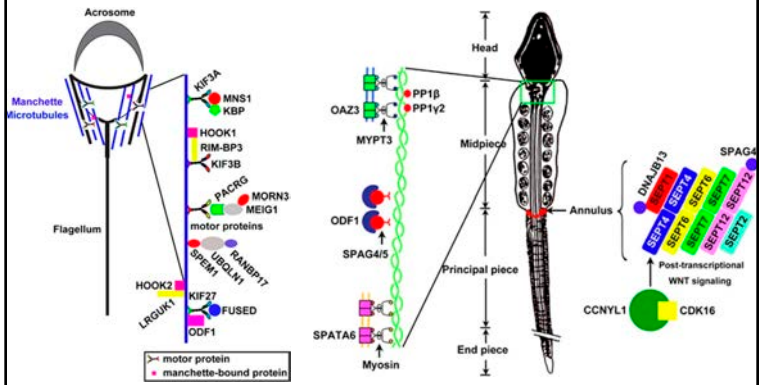
mTORC1/rpS6 and p-FAK-Y407 signaling regulate spermatogenesis: Insights from studies of the adjudin pharmaceutical/toxicant model.
Wang L, Li L, Wu X, Wong CKC, Perrotta A, Silvestrini B, Sun F, Cheng CY.
Semin Cell Dev Biol. 2022 Jan;121:53-62.



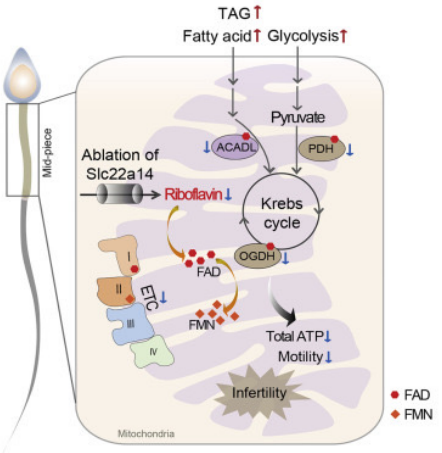
Blockade of GRTH/DDX25 Phosphorylation by Cyclic Peptides Provides an Avenue for Developing a Nonhormonal Male Contraceptive
Raju M, Kavarthapu R, Anbazhagan R, et al.
J Med Chem. 2021 Oct 14;64(19):14715-14727.



The control of male fertility by spermatid-specific factors: searching for contraceptive targets from spermatozoon's head to tail.
Cell Death Dis. 2016 Nov 10;7(11):e2472.
Chen SR, Batool A, Wang YQ, Hao XX, Chang CS, Cheng CY, Liu YX.



SLC22A14 is a mitochondrial riboflavin transporter required for sperm oxidative phosphorylation and male fertility
Kuang W, Zhang J, Lan Z, et al.
Cell Rep. 2021 Apr 20;35(3):109025.



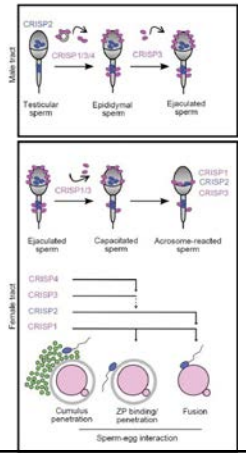
Highlights

- *Slc22a14* deficiency results in decreased sperm motility and male infertility
- *Slc22a14* ablation disrupts fatty acid β-oxidation and flavoenzyme activity
- Slc22a14 is a riboflavin transporter located at inner mitochondrial membrane in sperm

From the epididymis to the egg: participation of CRISP proteins in mammalian fertilization.

Da Ros VG, Muñoz MW, Battistone MA, Brukman NG, Carvajal G, Curci L, Gómez-Ellas MD, Cohen DB, Cuasnicu PS.

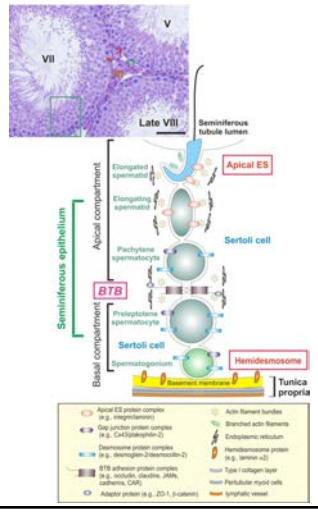
Asian J Androl. 2015 Sep-Oct;17(5):711-5.



The blood-testis barrier and its implications for male contraception.

Cheng CY, Mruk DD.

Pharmacol Rev. 2012 Jan;64(1):16-64.



Small-molecule inhibition of BRDT for male contraception.

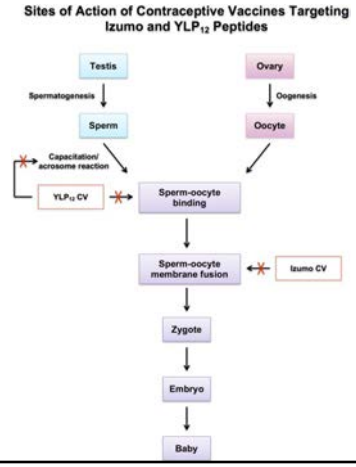
Matzuk MM, et al.

Cell. 2012 Aug 17;150(4):673-84.

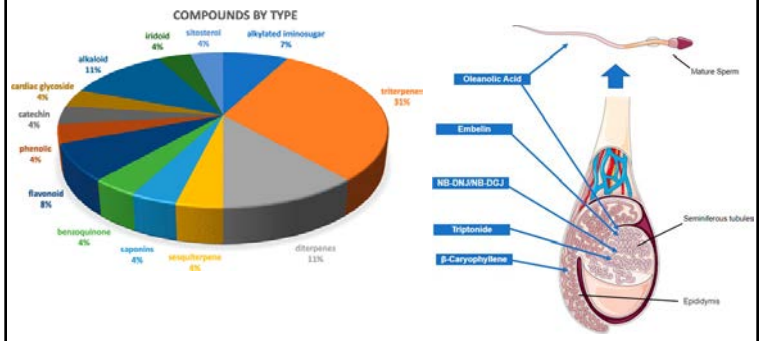
Vaccine for human contraception targeting sperm Izumo protein and YLP12 dodecamer peptide.

Naz RK.

Protein Sci. 2014 Jul;23(7):857-68.



Natural Products with Potential for Nonhormonal Male Contraception
Shunnarah A, Tumlinson R, Calderón AI.
J Nat Prod. 2021 Oct 22;84(10):2762-2774.



Triptonide is a reversible non-hormonal male contraceptive agent in mice and non-human primates
Chang Z, Qin W, Zheng H, et al.
Nat Commun. 2021 Feb 23;12(1):1253.

Abstract

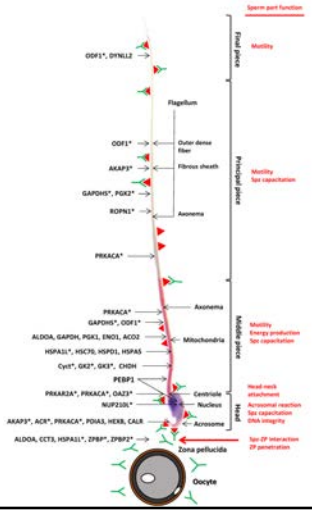
There are no non-hormonal male contraceptives currently on the market despite decades of efforts toward the development of "male pills". Here, we report that triptonide, a natural compound purified from the Chinese herb Tripterygium Wilfordii Hook F displays reversible male contraceptive effects in both mice and monkeys. Single daily oral doses of triptonide induces deformed sperm with minimal or no forward motility (close to 100% penetrance) and consequently male infertility in 3-4 and 5-6 weeks in mice and cynomolgus monkeys, respectively. Male fertility is regained in ~4-6 weeks after cessation of triptonide intake in both species. Either short- or long-term triptonide treatment causes no discernable systematic toxic side effects based on histological

Development of antifertility vaccine using sperm specific proteins.
Bandivdekar AH.
Indian J Med Res. 2014 Nov;140 Suppl:S73-7. Review.

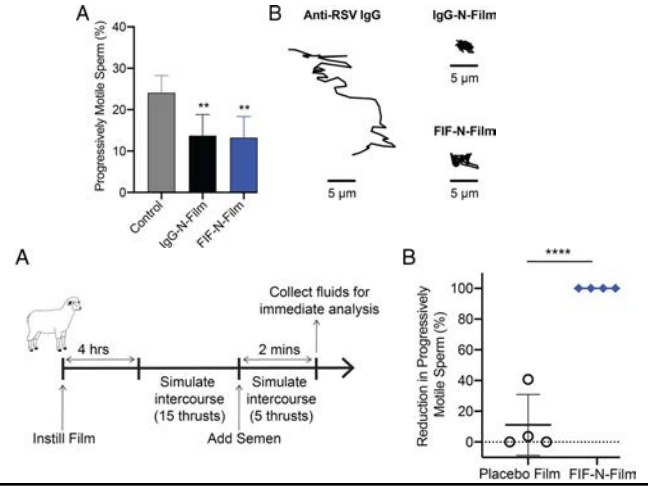
Table I. Antifertility effect of passive administration of antibodies to 80kDa HSA and its synthetic peptides

	Number of males fertile (% infertile animals)	Number of females fertile (% infertile animals)
Control	7 (12.5)	8 (0)
Ab 80kDa HSA	2 (75)	2 (75)
Ab peptide NT	3 (62.5)	2 (75)
Ab peptide 1	2 (75)	2 (75)
Ab peptide 2	4 (50)	4 (50)
Ab peptide 4	6 (25)	4 (50)

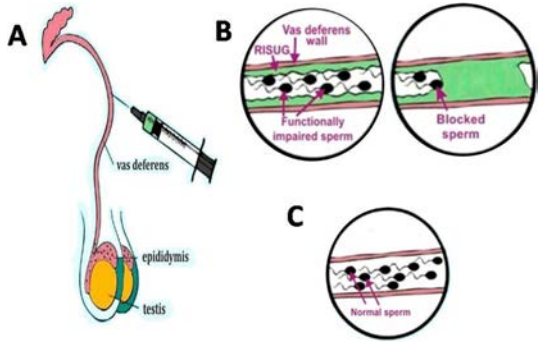
Identification of Arvicola terrestris scherman Sperm Antigens for Immune Contraceptive Purposes
Chorfa A, Goubely C, Henry-Berger J, et al.
Int J Mol Sci. 2021 Sep 15;22(18):9965.



Hexavalent sperm-binding IgG antibody released from vaginal film for development of potent on-demand nonhormonal female contraception
Shrestha B, Vincent K, Alison Schaefer A, et al.
Proc Natl Acad Sci U S A. 2021 Nov 30;118(48):e2107832118.



RISUG® as a male contraceptive: journey from bench to bedside.
Khilwani B, Badar A, Ansari AS, Lohiya NK.
Basic Clin Androl. 2020 Feb 13;30:2.



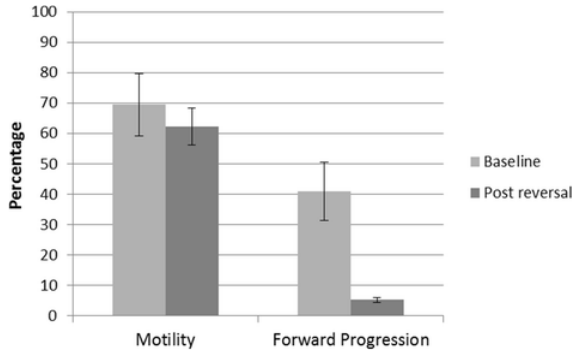
Contraception with RISUG® and functional reversal through DMSO and NaHCO3 in male rabbits.
Asian J Androl. 2017 Jul-Aug;19(4):389-395.
Ansari AS, Badar A, Balasubramanian K, Lohiya NK.

Table 3: Fertility record (percent of fertility) of male rabbits, prior to, and at various intervals following intravasal injection with RISUG and reversal with DMSO/$NaHCO_3$ (male mated with females in 1:2 ratio)

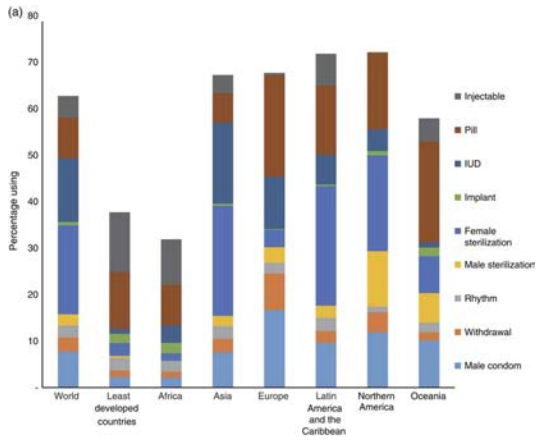
Mating schedule	*Group I*	*Group II*	*Group III*	*Group IV*	*Group V*	*Group VI*	*Group VII*
Preinjection	100	100	100	100	100	100	100
Vas occlusion (day)							
15	100	0	0	0	0	0	0
30	100	0	0	0	0	0	0
45	100	0	0	0	0	0	0
60	100	0	0	0	0	0	0
75	100	0	0	0	0	0	0
90	100	0	0	0	0	0	0
105-360	100				0	0	0
Reversal (day)							
15	100		0	0		0	0
30	100		0	0		0	0
45	100		0	0		0	0
60	100		0	0		0	0
75	100		0	0		0	10
90	100		20	0		60	50
105	100		60	60		70	90
120	100		80	90		90	90
135	100		100	90		90	100
150	100		100	100		100	100

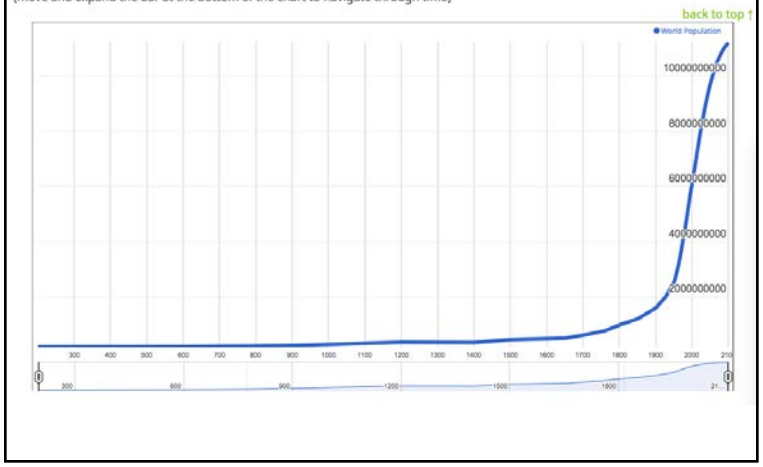
Group I: sham-operated control; Group II: vas occlusion with RISUG for 90 days; Group III: vas occlusion with RISUG for 90 days and reversal with DMSO; Group IV: vas occlusion with RISUG for 90 days and reversal with 5% $NaHCO_3$; Group V: vas occlusion with RISUG for 360 days; Group VI: vas occlusion with RISUG for 360 days and reversal with DMSO; Group VII: vas occlusion with RISUG for 360 days and reversal with 5% $NaHCO_3$. RISUG: reversible inhibition of sperm under guidance; DMSO: dimethyl sulfoxide; $NaHCO_3$: sodium bicarbonate

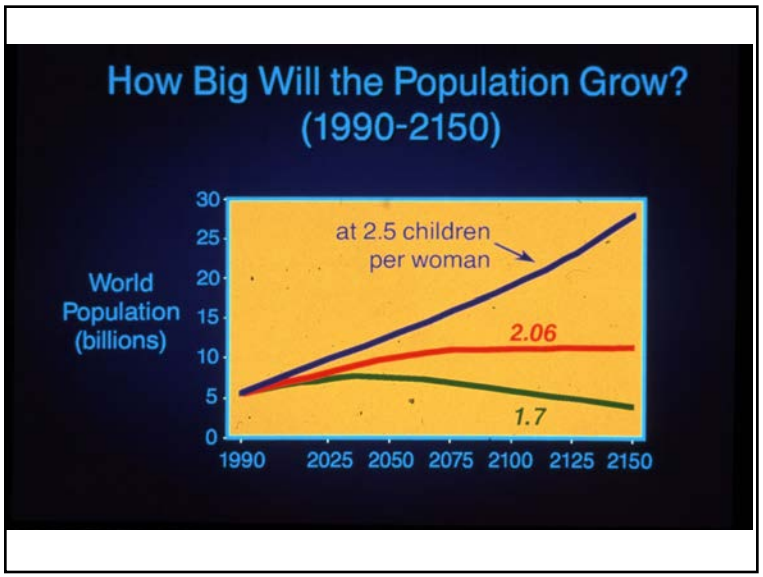
Reversibility of Vasalgel™ male contraceptive in a rabbit model.
Basic Clin Androl. 2017 Apr 5;27:8.
Waller D, Bolick D, Lissner E, Premanandan C, Gamerman G.



USE OF MALE METHODS OF CONTRACEPTION WORLDWIDE.
J Biosoc Sci. 2017 Sep;49(5):648-663.
Ross J, Hardee K.



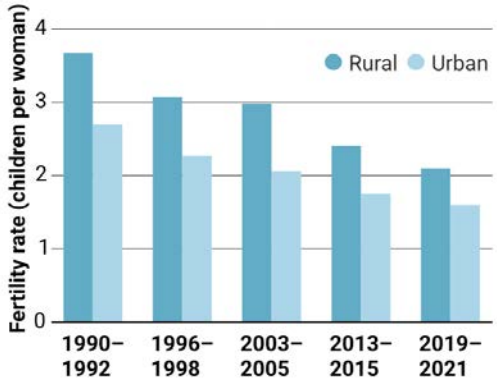




India defuses its population bomb.

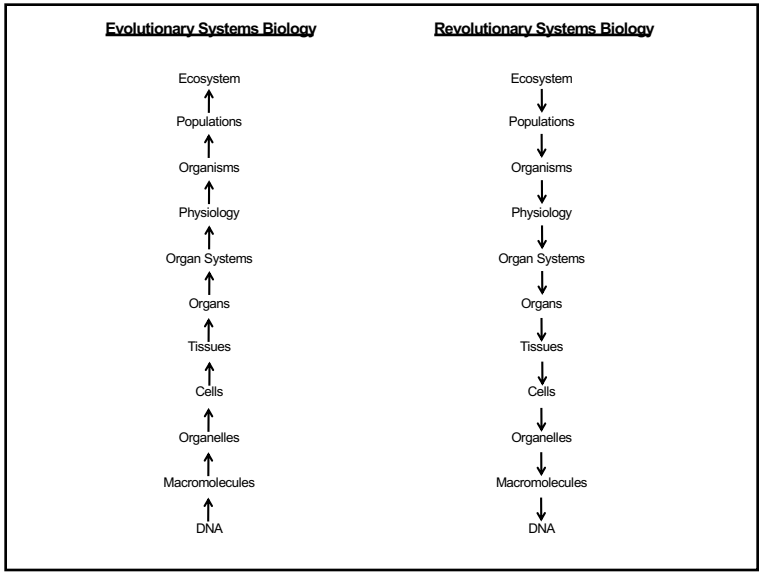
Pearce F.

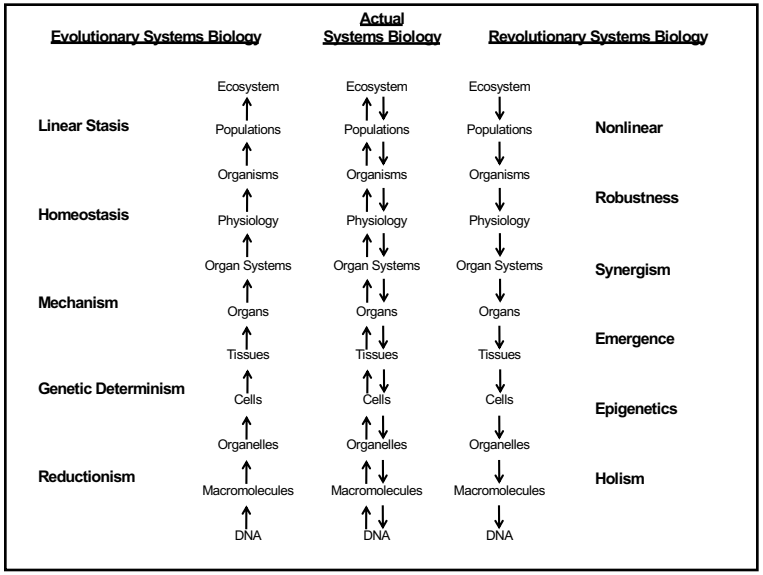
Science. 2021 Dec 17;374(6574):1422-1423.



Steadily shrinking families

Rural women in India tend to have more children than urban women,





"Systems Biology of Reproduction"

Spring 2022 (Even Years) – Course Syllabus
BIOL 475/575 Level Undergraduate/Graduate (3 Credit)
SLN: (475) – 05504, (575) – 05505
Time - Tuesday and Thursday 10:35 am-11:50 am
Course Lectures in person and and on Canvas/Panopto and Discussion Sessions in person and on WSU Zoom for all campuses
Room – CUE 418
Course Director – Michael Skinner, Abelson Hall 507, 335-1524, skinner@wsu.edu
Co-Instructor – Eric Nilsson, Abelson Hall 507, 225-1835, nilsson@wsu.edu
Learning Objective -
Current literature based course on the Systems Biology of Reproduction. Learning Systems approaches to the biology of reproduction from a molecular to physiological level of understanding.
Schedule/Lecture Outline –

January	11 & 13	Week 1	Systems Biology Introduction
	18 & 20	Week 2	Molecular/ Cellular/ Reproduction Systems
	25 & 27	Week 3	Sex Determination Systems
February	1 & 3	Week 4	Male Reproductive Tract Development & Function
	8 & 10	Week 5	Female Reproductive Tract Development & Function
	15 & 17	Week 6	Gonadal Developmental Systems Biology
	22 & 24	Week 7	Testis Systems Biology
March	1 & 3	Week 8	Ovary Systems Biology
	8 & 10	Week 9	Epigenetics and Transgenerational Gonadal Disease
	14 – 18	**Week 10**	**Spring Break**
	22 & 24	Week 11	Gametogenesis/ Stem Cells/ Cloning
	29 & 31	Week 12	Hypothalamus-Pituitary Development & Function
April	5 & 7	Week 13	Reproductive Endocrinology Systems
	12 & 14	Week 14	Fertilization & Implantation Systems
	19 & 21	Week 15	Fetal Development & Birth Systems
	26 & 28	Week 16	Assisted Reproduction/Contraception
May	3 & 5	Week 17	Exam or Grant Review