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Review Series

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As the world grows: contraception in the 21st century

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Retrospect

More than 700,000 maternal deaths, most in the developing world and related to causes associated with unintended pregnancies, occurred between 1995 and 2000; more than 400,000 of these deaths resulted from unsafe abortions (1). This indicates that existing contraceptives are either not available or not adequate. The need for safe and effective contraceptives has also been highlighted in two Institute of Medicine reports, published in 1996 and 2004 (2, 3). The 1996 report concluded that there is a need for contraceptive research and development; that contraceptive research and development need to be revitalized; and that there are scientific prospects that could revitalize the field (2). The 2004 report was more focused and addressed only two issues — facilitating translational research from contraceptive target identification to initial clinical studies and developing strategies for research success (3). Specific recommendations in the 2004 report were to utilize new "omic" technologies to assist in contraceptive target identification and validation; to improve the translational process with financial support and use of surrogate markers; to focus on druggable targets and tissue specificity; to seek to identify a priori rather than by serendipity other health benefits that the drugs might have in addition to contraception (e.g., prevention of breast and prostate carcinoma); and to recruit industry and a new generation of scientists to the cause.

There have been some advances in contraceptive development in the years since the first contraceptive revolution provided by the oral contraceptive (OC) pill regimen (4–6), e.g., the development of Copper T380A and levonorgestrel-releasing (LNG-releasing) intrauterine devices (IUDs) (7–9). However, most of the advances have, in fact, been developments of new delivery methods for existing and novel hormonal steroids, mostly acting by inhibition of ovulation. This has, to a large extent, been conflated by the fact that the length of time required to develop new methods has been substantially longer than many investigators and other experts in the field estimated (10, 11). Other problems hampering the development of new contraceptive methods include the fact that within the last two years, most large pharmaceutical companies involved in fertility regulation have withdrawn from the contraceptive research and development field. Thus, the outlook for the development of new contraceptives is grim and will depend solely on the public sector, where funding is also more constrained than in 1996. Realistically, development of a new contraceptive agent from target validation to the pivotal clinical trials can take up to 15–20 years and cost $100 million or more. These facts have been responsible for donor fatigue and some means has to be devised to reenergize the field (12).

Indeed, progress in the contraceptive development arena has been so poor that a recent report by the United Kingdom All Party Parliamentary Group on Population, Development and Reproductive Health (13) has concluded that the UN Millennium Development Goals cannot be met given the levels of population growth in the poorest countries. The Parliamentary Report also clearly indicated that claims advanced to refute the need for more contraceptive development — that birth rates are falling and good contraceptive methods are available now (14) — are not correct for many poor countries. In their 2004 article, Collumbien et al. (15) concluded that globally, maternal conditions stemming from unwanted births resulted in a loss of 98,000 lives and 4.5 million disability-adjusted life years. However, regional differences were large, with the effects being especially high in eastern and southern Africa. Non-use of available contraceptives is responsible for 90% of these unwanted births. A recent report from the Guttmacher Institute concluded that unmet contraceptive need ranges from 10%–12% in developing countries and that health effects and inconvenience are the main reasons for this non-use (16). For many women, existing contraceptives are therefore not acceptable or not available and thus in many cases are not used. Further, the importance of developing a diverse range of contraceptives acceptable to individual preferences is highlighted by the observa-

Nonstandard abbreviations used: Eppin, epididymal protease inhibitor; FSH, follicle-stimulating hormone; GAPDHS, GAPDH, spermatogenic; hCG, human chorionic gonadotropin; IUD, intrauterine device; LH, luteinizing hormone; LIF, leukemia inhibitory factor; LIFR, LIF receptor; LNG, levonorgestrel; OC, oral contraceptive; OR, odorant receptor; PC6, proprotein convertase 6; PEGylated, conjugated to polyethylene glycol; PRM, progesterone receptor modulator; RARα, retinoic acid receptor-α; TEX14, testis-expressed gene 14.

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tion that women in the United States who are neutral about or dissatisfied with their contraceptive method are more than three times more likely than women satisfied with their contraceptive method to have a gap of at least one month in a 12-month period during which they use no contraception despite being sexually active (17). There is very unlikely ever to be a contraceptive "silver bullet" that will suit all men or all women, irrespective of whether they are healthy and at what stage of their reproductive life cycle they are. This reinforces the need to develop various new (preferably nonhormonal) methods that are easy to use, safe, inexpensive, and sufficiently diverse in their methodology to be acceptable to individuals of different cultures and with different medical needs. Contraceptives that also provide protection against sexually transmitted infections, especially HIV, are urgently needed, as shown by the high rates of pregnancy and cervical infections in young sex workers in Madagascar (18).

To achieve these goals, it will be necessary to once again place population and family planning at the center of global efforts to improve reproductive health and fight poverty (19). As we discuss in this Review, we therefore need to know where we are: what is in the clinic, what is in the pipeline and at what stage, what needs to be done, and what are the key challenges? To close the gap between what contraceptive methods are available and what are needed, attention has turned to the new science of the omic revolution, which permits the identification of novel targets for contraception.

Contraceptives in the clinic and in clinical trials

Contraceptive methods for men and women can be divided into four main categories: steroidal methods, sterilization, vaccines, and barrier methods (physical and chemical). Tables 1–3 show contraceptive methods that have been recently approved and methods that are still in clinical trials.

Steroidal methods for women. Steroidal methods of contraception used by women prevent pregnancy by interfering with ovulation and the normal menstrual cycle (Figure 1). They can be oral or nonoral and can consist of an estrogen component combined with a progestin component or a progestin alone. The estrogen component of the contraceptive inhibits the release of follicle-stimulating hormone (FSH) from the anterior pituitary gland, thereby preventing follicular maturation. The progestin component inhibits the release of luteinizing hormone (LH) from the anterior pituitary, thereby preventing ovulation. The progestin also thickens cervical mucus, which impedes the movement of sperm into the uterus. Traditionally, combined OCs are taken for 21 consecutive days followed by 7 days of either no pills or placebo pills. When taken correctly and consistently, combined and progestin-only OCs are more than 99% effective at preventing pregnancy (20).

Recent modifications to OC regimens center on making the products more "forgiving" of incorrect use and on reducing uterine bleeding. The latter is usually an advantage in terms of convenience, but it can be medically important in women at risk for anemia. Both aims can be accomplished by eliminating the days on which no active pills are taken. This regimen avoids "escape ovulation," which can happen if a new pack of OCs is not started on time, and reduces the number of periods that women experience while taking OCs (21). Three OC products with modified regimens have entered the market in recent years: Seasonale, Seasonique, and Lybrel (Table 1).

OCs containing LNG can also be taken immediately after intercourse that was otherwise unprotected from conception: so-called emergency contraception. When taken within 72 hours of unprotected sex, they reduce the chance of pregnancy by 75%–89%. The main mechanism of action of such OCs is interference with ovulation, either by preventing or delaying luteal function (22). Steroidal antiprogestins are also effective (Table 1) (23).

Although efforts in the United States and other countries have centered on making emergency contraception more readily available, some new products are also being studied as alternatives to traditional steroidal methods in the hope that they have fewer side effects or are active for a longer period of time after unprotected intercourse (Table 1). For example, meloxicam is a COX-2 inhibitor that prevents follicular rupture and is being studied as an alternative emergency contraceptive that might expand the window during which emergency contraception is effective by 24 hours over current treatments (24, 25).

Nonoral forms of contraceptive steroids have been developed to eliminate the need for daily dosing. These include patches, vaginal rings, implants, injectables, and steroid-releasing IUDs (Table 1). There have been concerns about an increased risk of venous thromboembolic events for users of patch and ring approaches to nonoral steroidal contraceptives (26–28). However, these have not resulted in any of the approaches being withdrawn from the market, only labeling changes. The 6-rod implant Norplant, which was voluntarily withdrawn from the US market in 2002, has been replaced with better systems, Jadelle (two rods) and Implanon, a one-rod implant, and others are under study. Injectable steroidal contraceptives include progestin-only methods as well as combinations of progestin and estrogen. Progestin-only methods, given i.m. every 2–3 months, include medroxyprogesterone acetate and norethisterone enanthate. Combination injectables are available in at least 5 formulations and are given monthly. Although the progestin-only medroxyprogesterone acetate injectable Depo-Provera has been used worldwide for decades, there are concerns about its effect on bone mineral density (29–31). Mirena is an intrauterine system releasing 20 μg/d of LNG, which has proved to be highly effective and to have other health benefits, such as reduced uterine bleeding (9, 32).

Steroidal methods for men. Hormonal methods of male fertility regulation are based on the fact that exogenous testosterone can suppress spermatogenesis. This suppression is primarily achieved through feedback inhibition on the hypothalamic-pituitary-testicular axis, resulting in decreased release of pituitary gonadotropins FSH and LH. This then causes disruption of normal Sertoli cell function and endogenous testosterone production by testicular Leydig cells, respectively (33, 34). Normal sperm production is maintained by high intratesticular testosterone concentrations, and thus very low levels of intratesticular testosterone result in few or no detectable sperm, irrespective of circulating testosterone levels (35). Progestins also inhibit gonadotropin production and perhaps also exert a direct effect on the testis, thus providing a synergistic action (36). Numerous combinations of androgen and progestin have been tested in small sperm suppression studies; only one contraceptive effectiveness trial of a combination has been conducted (37).

A recent review of 30 studies of steroidal contraception in men found wide variation in the proportion of men who achieved azoospermia (38). The WHO has published the results of a large multicenter contraceptive study in which healthy men received 200 mg testosterone enanthate weekly by intramuscular injection for 6 months (Table 2) (35, 39). The low pregnancy rates were promising, but the frequent injection schedule was a problem. An

Table 1
Examples of steroid-based contraceptive products for women recently introduced in the clinic and in clinical trials

Product	Method of use	Effect and/or benefit	Stage of development
OCs			
Seasonale	84 tablets containing EE (an estrogen) and LNG (a progestin) and 7 placebo tablets	Menstrual periods are reduced from 1 per month to 1 every 3 months	In the clinic since 2003
Seasonique	84 tablets containing EE and LNG and 7 tablets containing 10 μg EE	Menstrual periods are reduced from 1 per month to 1 every 3 months. The 7 tablets containing 10 μg EE reduce breakthrough bleeding	In the clinic since 2006
Lybrel	28 tablets containing 90 μg of LNG and 20 μg of EE, to be taken continuously	No scheduled bleeding	In the clinic since 2007
Emergency contraceptives			
CDB-2914: VA-2914	50 mg taken within 5 days of unprotected intercourse	Synthetic selective PRM that binds the progesterone receptor but has no progestational activity	Phase III clinical trials (23)
Meloxicam	Dosage to be determined. Could be used alone or with LNG (24)	Nonsteroid that might expand the window during which emergency contraception is effective by 24 hours. Prevents follicular rupture even after the LH surge (25)	Inexpensive and widely available OTC; could be available OTC for emergency contraception
Nonoral steroidal contraceptives			
Ortho Evra patch	Releases 20 μg of EE and 150 μg norelgestromin daily	Prevents the need for daily dosing. In 2005, FDA required a new bolded warning that users are exposed to about 60% more total estrogen in their blood than women taking a daily OC containing 35 μg of estrogen because 1 of 2 studies found an increased risk of venous thromboembolic events (26, 28)	In the clinic since 2001
NuvaRing	Releases 150 μg etonogestrel and 15 μg EE per day	Prevents the need for daily dosing. In 2005, FDA required labeling to reflect an increased risk of thromboembolic and thrombotic disease	In the clinic since 2005
Nestorone ring	Releases 150 μg nestorone (a synthetic progestin not active when taken orally) and 15 μg EE per day	Prevents the need for daily dosing	Phase 3 clinical trial
Nestorone spray	Dosage to be determined; will be combined with estrogen	Avoids side effects common in oral dosing	Early trials
Nestorone rod	Single-rod uterine implant releasing nestorone for 2 years; daily transdermal spray	Prevents the need for daily dosing	Phase II trials of implant completed
Implanon	Single-rod uterine implant that contains 68 mg of etonogestrel released over 3 years.	Prevents the need for daily dosing	In the clinic since 2006
Depo-Provera	150 mg i.m. every 3 months	Prevents the need for daily dosing. In 2004, FDA required "black-box" warning regarding BMD as 2 studies suggested users could lose significant BMD (29–31). FDA warning states Depo-Provera should not be used for more than 2 years unless other contraceptives are inadequate. Other studies have generated different results, and the WHO does not advise restrictions.	In the clinic since 1992
LNG butanoate	10–50 mg i.m. every 3 months	Prevents the need for daily dosing. Thought to not affect BMD as Depo-Provera does	Early trials planned
Mirena intrauterine system	Contains 52 mg LNG; releases 20 μg/d	Less pain and bleeding than copper IUDs. Approved for only 5 years. Can cause steroid-related side effects such as acne and ovarian cysts (7, 9, 32).	In the clinic since 2000

BMD, bone mineral density; EE, ethinyl estradiol; OTC, over the counter.

improved formulation of testosterone undecanoate given once every two months suppressed spermatogenesis in a pilot study (40) and is to be tested in combination with norethisterone enanthate in a contraceptive effectiveness study (Table 2).

Table 2
Examples of steroid-based contraceptive products for men recently introduced in the clinic and in clinical trials

Product	Method of use	Effect and/or benefit	Stage of development
Testosterone enanthate	200 mg injected i.m. weekly for 6 months	Azoospermia in 65% of men in 6 months; only 1 pregnancy in 1,486 months (35). Recovery of sperm counts after about 4 months of no treatment (35). Second study determined that among men who achieved oligospermia, only 4 pregnancies occurred in 49.5 person-years of use, and among azoospermic men, there were no pregnancies in 230.4 person-years of use (39). Frequent injection schedule caused 5% to discontinue (39)	Proof of principle
Testosterone undecanoate	1,000 mg testosterone undecanoate and 200 mg norethisterone enanthate given in 2 separate i.m. injections every 8 weeks	Suppressed spermatogenesis (40). If found to be an effective contraceptive, both steroids will be formulated in 1 injection, which should increase acceptability	Contraceptive effectiveness study about to start

Sterilization. Nonsurgical (transcervical) methods of female sterilization offer lower cost, fewer complications, and faster recovery than surgical sterilization. However, the need for hysteroscopy affects their utility in developing countries (41). Products that can be used to achieve nonsurgical sterilization of women include liquids, coils, plugs, and chemical irritants (Table 3). For males, recent alternatives to vasectomy are aimed at improved reversibility (42) and include implants and gels (Table 3) (43–45).

Contraceptive vaccines. In the 1980s, there was much activity in the area of contraceptive vaccines, targeting antigens such as human chorionic gonadotrophin (hCG), FSH, and gonadotrophin-releasing hormone (GnRH) (46). However, due to lack of progress and funding as well as safety concerns, only a low level of effort on the hCG vaccine for women continues. This vaccine comprises a formulation of two novel poly(lactic-co-glycolic acid) microspheres that deliver a synthetic hCG peptide cosynthesized with a T cell epitope from tetanus toxoid (47). Contraceptive vaccines for men are still very much in the basic science phase, with the recently discovered epididymal protease inhibitor (Eppin) (48) providing a potential target antigen.

Barrier methods. Barrier methods are of two kinds: mechanical and chemical (Table 3). Often they are used together, as in the case of a diaphragm used with a spermicide. Efforts to improve mechanical barriers such as diaphragms and cervical caps have focused on easier fitting, ideally with one size that fits all users, use of nonlatex materials to avoid the problem of latex allergies, and easier insertion and removal. Traditionally used for contraception, cervical barriers are now being studied as a method to prevent sexually transmitted infections and HIV (49). However, a recent study in 4,500 women in South Africa and Zimbabwe showed that provision of a latex diaphragm with lubricant gel plus condoms provided no greater protection against infection with HIV than provision of condoms alone (50).

The Reality female condom has been in use to some degree in many countries since 1992 (Table 3) (51–54). Limitations to its more widespread use center on cost and use characteristics. Reuse studies have been done to address the former and, although reuse is not recommended, the WHO has developed a protocol for cleaning female condoms with a bleach solution, permitting reuse five times. Washing with soap and water is also effective (55). Use of synthetic latex should also reduce costs. Problems of acceptability have led to a wide variety of new designs. At least two are variations on a replaceable-condom-in-a-panty, which is felt to have a more acceptable appearance than the outer ring of the original female condom. Others include the Reddy latex female condom and PATH's woman's condom (Table 3) (56).

Nonoxynol-9 is a moderately effective spermicidal chemical contraceptive, but frequent use has been suggested to increase the risk of becoming infected with HIV compared with placebo (57). This negative effect might be due to disruption of the integrity of the cervicovaginal mucosa and induction of an immunoinflammatory response facilitating infection with HIV (58, 59). Recent work has focused on alternative products, most of which display both contraceptive and microbicidal activity without mucosal irritating properties (Table 3) (60). Cellulose sulfate 6% vaginal gel was effective as a vaginal contraceptive but failed to protect women against HIV infection (61–63).

How the omic revolution has had an impact on contraceptive development

The last 25 years have seen remarkable developments in the areas of genomics and proteomics as well as the generation of transgenic models for the study of reproduction (64). Several mammalian genomes, including the human, rhesus macaque, mouse, and rat, have been almost completely sequenced. With new advances in DNA sequencing technology, multiple genomes are being sequenced each year at amazing speed. Transcriptomes of individual cells, tissues, and organisms have been quickly deposited into databases and include not only messenger, transfer, and ribosomal RNAs, but also microRNAs, siRNAs, and PIWI-interacting RNAs. In parallel with these advances, new technologies permitting the large-scale characterization of complex protein mixtures (proteomics), and the integration of these proteins into known pathways (systems biology) have had a major impact on the potential for contraceptive development, especially in males (65). In addition, the advent of metabolomic technologies enables us to profile the metabolic processes that are critical to reproductive function. Integration of these metabolic data with the proteome and transcriptome will revolutionize our understanding of how the reproductive system works and, at the same time, identify and characterize key targets for novel contraceptives.

Challenges to using genomic information. Recommendation 1 of the 2004 Institute of Medicine report (3) was to "Identify and characterize all genes and proteins uniquely or preferentially expressed in the

Table 3
Examples of nonsteroidal contraceptive products recently introduced in the clinic and in clinical trials

Product	Method of use	Effect and/or benefit	Stage of development
Nonsurgical female sterilization			
Ovabloc	Liquid siloxane is inserted into the tubal orifice, then polymerizes into a plug		Not approved
Essure	A small metal coil is placed into each fallopian tube through the cervix using a catheter. Once in place, the device elicits tissue growth to form an occlusion		In the clinic since 2002
Quinacrine	Chemical tubal occlusion. 7 pellets placed high in the uterus using a modified IUD inserter; these dissolve and cause scarring of the opening of the fallopian tube		Abandoned due to carcinogenicity concerns (171, 172)
Vasal occlusion			
Intravas device (SHUG)	Two types: a pair of silicone plugs and urethane tube lined with nylon sieve	Trap sperm but allow fluid in the vas to pass through, reducing likelihood of congestive epididymitis. Double-plug design did not completely block sperm transport through the epididymis, failing in 3 of 30 men (43)	Not approved
RISUG	Styrene maleic anhydride in a solvent vehicle of dimethyl sulfoxide gel injected into the vas	Partially occludes vas and has direct toxic effect on sperm for at least 1 year (44). May be reversible with second injection (45)	Phase III clinical trials in India
Barrier methods			
SILCS	Silicone cervical diaphragm	Available in 1 size believed to fit most women (173, 174)	Phase III contraceptive effectiveness trial
FC2	Female condom made of synthetic latex	Should cost less than original female condom (52). Performance and acceptability of original female condom and FC2 were comparable in South African trial (53, 54).	Has received CE marking for product and manufacturing but is not approved in US
Reddy latex female condom	Soft polyurethane sponge aids insertion	Appeared more acceptable than original female condom in Indian trial (56).	Not approved in US
PATH's woman's condom	4 small foam dots on outside of inner ring adhere to vagina to hold condom in place	Closed end of condom is gathered into gelatin-based capsule that facilitates insertion, then dissolves after use	Phase I trial completed
UsherCell	6% cellulose sulfate vaginal gel to be inserted before intercourse	Inhibits sperm function and egg binding (61). Has shown promise in contraceptive efficacy trials (62), but 2 HIV prevention trials were stopped for lack of efficacy and possible increased risk of infection, which in the final intent-to-treat analysis proved not significant (63)	Development on hold
BufferGel	Carbopol-based gel to be inserted before intercourse	Immobilizes spermatozoa by lowering pH (175). Has shown promise in contraceptive efficacy trials (176) and is being evaluated for HIV prevention	Phase III contraceptive trial of BufferGel with diaphragm completed; phase III HIV prevention trial underway

RISUG, Reversible Inhibition of Sperm Under Guidance; CE marking, mandatory conformity mark on products placed on the single market in the European Economic Area (EEA).

testis, ovary, and reproductive tissues; and define the genetic and protein networks in cells relevant to reproduction, including construction of a protein interaction map for the sperm and the egg." Despite all the progress in generating omic information, there are more than 1,000 reproductive tissue–expressed genes for which there is minimal information available about the full-length mRNA, the protein product of the gene, and the protein localization (66–68). The actual number might actually run considerably higher, since published data indicate that 4% of the mammalian genome (i.e., more than 2,300 genes) comprises genes that are specifically expressed in the male germline during or after the completion of meiosis (66). Proteomic analysis of human, rat, and mouse spermatozoa has demonstrated that over 1,000 proteins are present in each of these cell types (65). Since many of the proteins are considered "novel" (i.e., do not con-

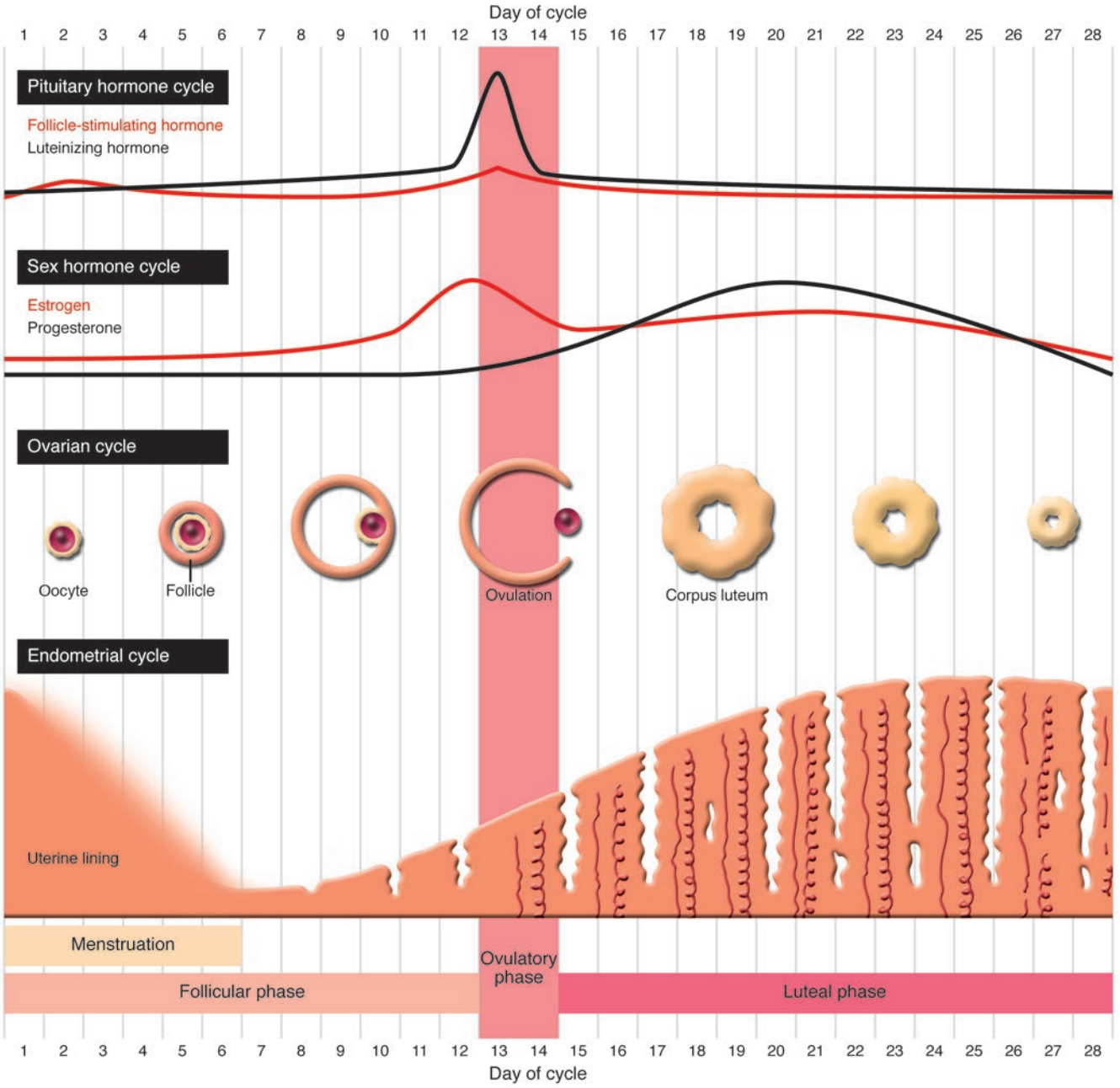

Figure 1
The human female menstrual cycle. The human female menstrual cycle is divided into several phases, which vary in length among women and among cycles; average times are indicated. The first phase of the menstrual cycle is the follicular phase, and it begins the day that menstrual bleeding starts. A decrease in the levels of estrogen and progesterone triggers the top layers of the thickened endometrium to break down and be shed, resulting in bleeding. Concomitant with this, levels of FSH increase very slightly, stimulating the development of several oocyte-containing follicles. FSH levels subsequently decrease and only one or two follicles continue to develop. The developing follicles release estrogen, and this initiates thickening of the endometrium, something that continues throughout the rest of the menstrual cycle. The second phase of the menstrual cycle is the ovulatory phase. It begins at approximately day 13, when levels of LH and FSH increase dramatically; levels of estrogen also peak at this time, and levels of progesterone begin to increase. The high levels of LH stimulate ovulation. The final phase of the menstrual cycle is the luteal phase. During this phase, levels of LH and FSH decrease and the ruptured follicle forms the corpus luteum, which produces large amounts of progesterone. Progesterone modifies the endometrium so that it is receptive to implantation of an embryo if fertilization has occurred. In the absence of fertilization, the corpus luteum degenerates and the loss of progesterone production, combined with decreased levels of estrogens, initiates a new menstrual cycle.

tain domains that match known proteins), protein localization, protein interaction networks, and spatiotemporal expression and function cannot be inferred, meaning that they remain to be explored as potential contraceptive targets.

A common way to determine the function of a gene is to generate a knockout mouse. If a knockout of a gene in mice results in a fertility defect (and more importantly, infertility), then this is a proof of principle that blocking the function of the protein product with a small molecule inhibitor would have a contraceptive effect. Indeed, recommendation 3 of the 2004 Institute of Medicine report (3) was to "Validate existing and emerging contraceptive targets by using forward and reverse genetic approaches with model organisms." More than 200 mouse models have been created or identified in which there is a fertility phenotype (reviewed in refs. 64 and 69). Although this approach has been used on an individual gene basis by specific laboratories, government funding is limited for the production of a knockout mouse of a gene that encodes a protein with no known functional domains. Thus, hundreds of excellent potential contraceptive targets lie dormant because bioinformatics cannot categorize them. Currently, the only way to approach this is to produce antibodies to the novel protein, thereby gaining the wealth of information that is needed to identify reproductive tissue–enriched contraceptive targets that can be further validated using gene-deletion approaches.

Proteomics and systems biology. Detailed proteomic analysis of human, rat, and mouse spermatozoa have now been completed and provide a rational basis for selecting targets for contraceptive development (65). Bioinformatic analyses of these data suggest that approximately 2% of the sperm proteome is amenable for drug-target validation, with several leading candidates currently in the pipeline for further development, such as the odorant receptors (ORs) and sperm thioredoxins mentioned below (70). In the case of the human sperm proteome, there are at least six seven-pass transmembrane GPCRs, six tyrosine kinase receptors, a tyrosine phosphatase receptor, glutamate-gated ion channel receptors, transient receptor potential cation channels, and a nongenomic progesterone receptor-transmembrane, all of which are potential contraceptive targets, by virtue of being surface receptors amenable to pharmacological intervention (65). The examples given below are being actively pursued from a contraceptive point of view. Of the GPCRs present in human spermatozoa, some of the most interesting from a contraceptive point of view are the ORs, which seem to play key roles in the regulation of sperm movement and chemotactic behavior (ref. 71; see below). Another interesting group of molecules from a contraceptive point of view identified in the sperm proteome are the testes-specific thioredoxins, which are thought to be involved in the redox regulation of sperm function (72) and specifically the control of sperm capacitation and motility.

Challenges to using proteomic information. Although proteomics might provide us with a valuable platform for identifying potential targets for contraceptive development, a clear bottleneck exists in the validation of these leads. Traditionally, the production of knockout mice was used to determine whether or not a particular gene product is essential for fertility. However, this approach has proven unexpectedly challenging. Key proteins found within human spermatozoa that might provide contraceptive targets, including some GPCRs (for example, the ORs; ref. 71), do not possess orthologous proteins present in the mouse genome, making it virtually impossible to move forward with any confidence. The reverse is also apparently true. Further, although putative candidates can be found in the mouse proteome, paralogous proteins (more than one orthologous protein) can be present within the human sperm proteome, creating redundancies that make validation of a particular target extremely difficult. One approach to circumvent this problem is to target a family of proteins, creating a compound directed toward two or more similar proteins known to be present in spermatozoa. However, the validation of this approach still requires the production of knockout mice; only in this case, multiple genes have to be disrupted to provide meaningful evaluation of a given contraceptive approach.

It is clear that careful target identification is a critical component of the contraceptive discovery process. In the context of male fertility control, it has been argued that the posttesticular maturation of spermatozoa in the epididymis and female reproductive tract might provide a better source of contraceptive targets than spermatogenesis in terms of the safety, reversibility, and speed-of-onset of the contraceptive effect (73). The posttesticular remodeling of spermatozoa exclusively involves posttranslational modification of existing proteins as a result of such key processes as phosphorylation, proteolysis, and glycosylation. New technologies in comparative proteomics should be extremely valuable in characterizing these posttranslational modifications and identifying specific targets for further development as contraceptive agents. For example, by focusing on changes in the phospho-proteome it should be possible to highlight kinase- and phosphatase-dependent changes, which should be druggable. In a recent analysis of this type on rat spermatozoa, 9 proteins were found to become phosphorylated during capacitation (R.J. Aitken and M.A. Baker, unpublished observations). The ultimate purpose of such analyses is to generate a short list of sperm-specific, functionally important, druggable targets that can then be taken forward for validation.

New leads for contraceptives

The foregoing discussion has delineated the power of genomic and proteomic studies to identify thousands of reproductive tissue targets for future contraceptives. Functional contraceptive-relevant data for many of these targets is currently lacking (i.e., gene knockouts or knockdowns have not confirmed an important role for many of these proteins). The information in Table 4 includes a selection of the most interesting targets that are presently subject to intense exploration.

Gametogenesis. Although much is known about the key regulatory molecules involved in both oogenesis and spermatogenesis (69), only a few present themselves as suitable targets for contraception (Table 4) for many reasons (e.g., targeting of some proteins expressed outside the reproductive axis may cause unwanted side effects or others may not be feasible druggable targets). During the process of spermatogonial cell differentiation, male germ cells develop a unique structure called the intercellular bridge (74, 75). These intercellular bridges interconnect and link all subsequent male germ cells during mitotic and meiotic divisions. Thus, by the time haploid germ cells are formed, there is a clonally derived syncytium of up to approximately 1,000 germ cells that are interconnected by intercellular bridges. Although proteins involved in the formation of intercellular bridges in the fruit fly have been identified, essential proteins involved in the formation of the mammalian intercellular bridge have not been identified until recently (74, 75). Testis-expressed gene 14 (TEX14) was shown to localize specifically to the intercellular bridge throughout spermatogenesis (74) and convert some midbody matrix proteins (i.e., the centralspindlin complex) into stable intercellular bridge components (75). Absence of TEX14 in mice not only disrupts

Table 4
Targets for potential contraceptives based on an infertility phenotype induced by gene knockout or antibody or small molecule inhibition

Target	Protein family	Role	Reference
TEX14	Kinase-like domain	Component of germ-cell intercellular bridge	74
Occludin	Occludin	Calcium-dependent cell adhesion molecule in tight junctions	78, 81
RARα	Steroid-hormone receptor superfamily; retinol-binding	Spermatid maturation	85
GAPDHS	Glycolytic enzyme	Sperm motility	101
CatSper	4 subunit calcium channel	Hyperactivated motility	103
Eppin	Kunitz-type trypsin inhibitor; defensins	Hydrolysis of semenogelin	48, 121
LIFR	Cytokine receptor	Blastocyst implantation	141
IL-11 receptor	Cytokine receptor	Decidualization	153
Leptin receptor	Cytokine receptor	Blastocyst implantation	158
PC6	Serine protease	Blastocyst implantation	167

the intercellular bridge but also causes sterility (74). Since TEX14 and the intercellular bridge are critical for fertility in males, protein components of the intercellular bridge, including TEX14, are potential contraceptive targets.

Recent studies have shown that the chemical Adjudin targets protein complexes (e.g., integrin/laminin and cadherin/catenin) at the apical ectoplasmic specialization, which is restricted to the interface between Sertoli cells and elongating spermatids (step 8 spermatids and beyond) in the rat testis. This induces premature exfoliation of sperm from the seminiferous epithelium by causing anchoring junction disruption between Sertoli cells and spermatids (76, 77). This results in a lack of mature spermatozoa in ejaculates, making them unable to fertilize oocytes (76). The effects of Adjudin on male fertility were reversible (76). Due to low oral bioavailability, large doses of Adjudin were required to produce this contraceptive effect, but they also caused liver toxicity, indicating a narrow therapeutic margin (76). FSH receptors are solely present on Sertoli cells in mammals, and so this problem of lack of bioavailability was overcome by using a recombinant, minimally hormonally active mutant FSH molecule to target Adjudin to Sertoli cells. Conjugation of Adjudin and the FSH mutant produced a molecule that, when administered i.p., caused infertility at a dose of Adjudin 100,000 times lower than had been needed orally for the unconjugated Adjudin (77).

Tight junctions create highly selective diffusion barriers that prevent the free passage of molecules and ions. Occludin, a four-pass integral plasma-membrane protein is a functional component of these barriers in rodents (78, 79). Combination of the same FSH mutant used to target Adjudin to Sertoli cells with a 22–amino acid peptide of occludin that is known to disrupt the blood-testis barrier in vivo (80) produced a molecule that bound to the FSH receptors on the Sertoli cells with negligible hormonal activity (81). Injection of this conjugate i.p. in rats caused reversible disruption of the blood-testis barrier and partial germ cell loss from the testis without compromising tight junctions in epithelia of other organs (81). This novel targeting vector provides a specific way of delivering contraceptive molecules to the testis. However, at this point in development, the need to administer the conjugate i.p. makes it impractical for regular contraception. Furthermore, the costs of synthesizing the recombinant FSH mutant make it prohibitively expensive for male contraception. Alternate expression and delivery systems need to be investigated. If these problems can be overcome, occludin could prove to be a viable contraceptive target (Table 4).

The need for dietary retinol (vitamin A) for normal spermatogenesis is well recognized (82–84). Mice lacking retinoic acid receptor-α (RARα), a critical protein for transducing retinoid-mediated signaling, are infertile due to multiple specific defects in spermatogenesis (85). This includes a failure of spermatids to align and be released into the lumen, presumably reflecting aberrant orientation to the Sertoli cells (85, 86). BMS-189453 is a synthetic retinoid that acts as a panantagonist of all three RARs (RARα, RARβ, and RARγ). Rats and rabbits treated orally with low doses of BMS-189453 for only one week exhibited profound testicular toxicity within one month after cessation of treatment and minimal systemic toxicity (87). In contrast to the reversibility observed upon restoration of vitamin A to the diet, the effects of the pan-RAR antagonist were reported to be irreversible (87). However, recent experiments with lower doses of this antagonist have resulted in male infertility that was reversible (D. J. Wolgemuth, personal communication). These results suggest that it might be possible to identify other related small molecules specific for RARs that would interfere with spermatogenesis and yet be reversible.

Gamete transport. Factors influencing sperm transport through the female reproductive tract are regulated to ensure the best chance for motile, healthy spermatozoa to fertilize the oocyte (88). After maturation in the epididymis, many millions of sperm are ejaculated, but only a few thousand reach the site of fertilization in the fallopian tube. The cervix and the uterine lumen pose barriers that must be overcome (89). Sperm transport through the cervix is affected by the state of the cervical mucus. Midcycle mucus under the influence of estrogen is thin and watery and composed of macromolecular fibrils arranged to form micelles large enough to permit sperm passage. In contrast, under progesterone dominance, the mucus is thicker, contains less water, and does not form micelles, thus making it less suitable for sperm passage (90). Creation of such hostile mucus is thought to be the major mechanism of action of progesterone-only contraceptive pills. Endocervical expression of a gel-forming mucin, MUC5B, and of a major membrane-spanning mucin, MUC4, seems to produce mucus, permitting sperm passage through the cervix (91). Increasing progesterone blood levels diminish such expression (91). An agent that could reliably achieve such hostile mucus through a local, nonhormonal, mechanism would be an interesting contraceptive target, but further studies to elucidate the mechanism controlling hormonal regulation of cervical mucus are still needed (91).

It has been known for a long time that men treated with thioridazine, an antipsychotic drug, or phenoxybenzamine, an anti-

hypertensive agent, become infertile due to failure of ejaculation, even though orgasm is normal (92, 93). It was suggested that this contraceptive effect of phenoxybenzamine was due to a selective blockade of α-adrenoreceptors in longitudinal but not in circular muscular contractions of the vas deferens (94), a concept that is supported by in vitro observations (95). Work is underway to identify suitable small molecules that could act specifically on the longitudinal muscles of the male reproductive tract. Such an agent would have great attractiveness as a male contraceptive since it could be taken orally only hours before need and its effects could be completely dissipated within 24 hours.

The glycolytic pathway is modified extensively in mammalian spermatozoa and includes multiple isozymes that are not expressed in somatic tissues. GAPDHS (known as GAPDH-2 in humans), phosphoglycerate kinase 2, and two aldolase isozymes (ALDOART1 and ALDOART2) are encoded by genes expressed only during spermatogenesis (96–99). Other novel isozymes in sperm, such as hexokinase 1-S, are generated by alternative splicing events that occur only in the male germ line (100). GAPDHS and several other glycolytic enzymes are tightly bound to the fibrous sheath, a cytoskeletal structure in the principal piece of sperm flagella (98). Consistent with these findings, glycolysis in mature spermatozoa is only observed in the principal piece. Generation of mice lacking GAPDHS demonstrated that this protein is essential for sperm motility and male fertility (101). In the rat, the *GAPDHS* transcript is found only in the testis and first appears about day 29 after birth (102). Transcripts were only found in round and condensing spermatids, demonstrating haploid expression of the *GAPDHS* gene (102). These findings suggest that inhibition of human sperm GAPDHS, either in the male reproductive tract or the female reproductive tract (e.g., through a small molecule applied vaginally), might inhibit sperm motility and prevent fertilization (Table 4).

Motility is required for normal fertility. When sperm enter the alkaline environment in the upper portion of the female reproductive tract, they exhibit a specialized pattern of motility termed *hyperactivation* by which the flagellar wave generates high levels of forward thrust (88). This hyperactivated motility requires Ca^{2+} entry (103). Among the Ca^{2+} channels that have been identified in sperm are the CatSpers. Four sperm-expressed CatSper proteins have been found to date (104, 105). It seems that Ca^{2+} influx into the principal piece of the sperm via the CatSper (CatSper1–CatSper4) channels initiates hyperactivated motility and a tail-to-head propagation, leading to an increase in NADH (106). Absence of any one of the four CatSpers in mouse sperm results in an identical phenotype, i.e., no effect on initial sperm motility, but abolition of hyperactivated motility, resulting in infertility (103, 105, 107–109). Consistent with these findings and the observation that the localization of each of the CatSper proteins in the principal piece is similar (107–109), the four CatSper proteins seem to be subunits of the same cation channel. In addition, infertile men who lack sperm motility exhibit decreased *CATSPER* gene expression compared with men infertile for other reasons (110). Hence, blockade of the spermatozoa CATSPER cation channel in the male or female tract might be expected to have a contraceptive effect (Table 4).

Although one would expect ORs to be unique to the sensory neurons of the olfactory epithelium, a number are also expressed in mammalian sperm (111). Studies over many decades have suggested that sperm can exhibit chemotaxis in response to specific chemicals and the products of ovulation (112). However, the connection between ORs and chemotaxis was only made more recently (111). Signaling through a specific human testicular OR, hOR17-4, caused both chemokinetic and chemotactic responses by sperm in vitro (71, 111). Bourgeonal was identified as an agonist that could stimulate these responses, whereas undecanol functioned as an antagonist of these effects (71, 111). Signaling through additional ORs seems to stimulate specific responses in sperm (71). The endogenous ligands in the female reproductive tracts that cause these responses are likely to be reported shortly. These studies indicate that agonists and/or antagonists of one or more OR signaling pathways might be excellent contraceptives that would be effective in the female reproductive tract.

Fertilization. Although considerable progress has been made in identifying egg and sperm components involved in each of the steps that lead to fertilization, several issues remain unresolved or contentious. For example, although numerous candidates have been reported (113, 114), the precise nature of the sperm proteins that bind zona pellucida protein 3 (ZP3) on the oocyte is unclear. Similarly, there is a great deal of confusion as to which sperm and egg proteins are actually involved in binding and fusion of gametes (115).

One recent lead is Eppin (48). In the human, sperm rapidly begin to swim into the cervical canal after ejaculation (116). Human semen coagulates as a loose gel that is enzymatically degraded within one hour, enabling the sperm to move more freely along the reproductive tract toward the oocyte (117). The predominant structural components of the vesicular gel are semenogelin I and semenogelin II, which are degraded by the serine protease prostate-specific antigen (PSA) (118). Eppin, which originates from Sertoli and epididymal epithelial cells, binds semenogelin and inhibits the activity of PSA and therefore the hydrolysis of semenogelin (119, 120). The C-terminal portion of Eppin is structurally homologous to the Kunitz-type trypsin inhibitor, and the N-terminal has structural similarity to defensins (121). Human sperm have receptors for Eppin, and antibodies specific for Eppin cause infertility in male monkeys, which is reversed as antibody levels fall (48). It is suggested that Eppin-specific antibodies bound to Eppin on the sperm surface block the binding site for semenogelin (121). Development of small molecules that would block this binding site might be expected to be contraceptive (Table 4).

The high rate of HIV infections acquired through sexual activity warrants the development of novel compounds displaying contraceptive and microbicidal anti-HIV properties. Some of the molecules and mechanisms used by sperm to fertilize the egg are similar, if not identical, to those used by HIV to infect host cells (122). An example of common structures is the lipid membrane surrounding the spermatozoan and the HIV core (122). Disruption of the architecture of the lipid membrane by surface-active compounds exerts both spermicidal and virucidal activity (123). A more specific alteration of lipid rafts by β-cyclodextrins also has similar effects (124). During fertilization and infection, both sperm and HIV interact with their target cells through chemical charges, hydrophobic forces, and carbohydrate recognition. Anionic polymers such as cellulose sulfate and polystyrene sulfonate inhibit sperm and HIV cell binding and have been shown to have contraceptive effects (Table 3). Since some of the molecules involved in the interaction between sperm and oocytes are also used by other pathogens to infect their target tissues, polyanions exert broad antimicrobial activity as well as contraceptive effects.

During fertilization and infection, sperm and HIV, as well as other microbes, use signal transduction molecules and mechanisms such as adenyl cyclase/cAMP-dependent kinase, calcium, and tyrosine phosphorylation, whose inhibition has been shown to impair sperm function and HIV replication (122). These commonalities at the level of sperm and HIV structure, cell binding and fusion processes, and signaling pathways therefore provide the biological framework to develop bifunctional inhibitors with both antimicrobial and contraceptive properties (122).

Implantation: steroidal approaches. The search for agents that work postovulation up to and including blastocyst attachment to the endometrium has been vigorously pursued for over 50 years. However, the animal models used in most studies were rats or mice, which eventually proved to be unsuitable, since they require ovarian estrogen to initiate the process of implantation, whereas primates do not (125, 126). Hence, selective estrogen receptor modulators (SERMs) will not be contraceptives in women. However, the discovery of progesterone receptor modulators (PRMs), which are steroids with antiprogestational activity (as opposed to progestational activity), has provided a different approach to inhibition of ovulation and implantation than that utilized by current progestin-based OCs. For example, the PRM mifepristone can terminate pregnancy in women when taken orally after a missed menses, especially when combined with a prostaglandin, which progestins cannot (127). In addition, administration of 200 mg mifepristone alone on day LH plus 2 prevented pregnancy in all but 1 of 157 cycles in fertile women, resulting in a Pearl index of 7.6 (128). Since, in this case, the drug was administered after ovulation, it must have exerted its effect on the fallopian tube or endometrium (128). In contrast, oral daily doses of 2 or 4 mg of mifepristone inhibit ovulation and menstruation in most cycles in women, while maintaining follicle development and estradiol levels (129). No pregnancies were observed in 59 women over 200 months of daily exposure (129). This regimen has not become available, since the toxicology done on mifepristone is only adequate to support infrequent use for abortion or emergency contraception. VA2914, another PRM, has also been studied for its potential as a daily OC pill and been found to inhibit ovulation and induce amenorrhea in over 80% of women (130).

Implantation: nonsteroidal approaches. The failure of random screening to produce viable leads for anti-implantation agents led to the need to select targets for which proof of principle could be demonstrated. Microarrays of endometrial genes have permitted assessment of which genes are upregulated and which downregulated during the window of endometrial receptivity for implantation (131–137). Although there are similarities in gene profiles generated by different laboratories, there are also surprising discrepancies, and two recent papers have concluded that the key molecules and mechanisms regulating endometrial receptivity have yet to be elucidated (138, 139). In reality, many of the genes of current interest were being worked upon before the advent of microarrays. Some possible endometrial targets are described below. All of them raise questions as to specificity to the reproductive tract, but results from animal experiments (knockout mice or inhibition of action by antibodies or peptides) have not, so far, shown reason for concern. However, this is an area that requires vigilant monitoring, and appropriate toxicology tests will need to be conducted as research develops.

Leukemia inhibitory factor (*Lif*) mRNA appears in the endometrial glands of the mouse uterus on day 4 of pregnancy and pseudopregnancy but is absent in nonpregnant mice (140). In mice lacking LIF, although males were fertile, females were infertile due to failure of blastocysts to implant in the uterus (141). LIF protein is upregulated by estradiol in the mouse uterus but by progesterone in rabbits (142). In women, the situation is not so clear-cut. In nonconception cycles, both mRNA and protein are low or absent during the follicular (proliferative) phase but rise in the glands and luminal epithelium during the luteal (secretory) phase (143, 144). LIF signals through a high-affinity receptor comprised of LIF receptor (LIFR) and GP130, which mediates signal transduction through the JAK/STAT pathway (145, 146). GP130 is also a component of a number of other cytokine receptors, including those for IL-6, IL-11, ciliary neurotrophic factor, and oncostatin M (147). In contrast to *Lif*-deficient mice, germline deletion of the gene encoding LIFR is lethal (148). Instillation of either polyclonal or monoclonal antibodies against recombinant human LIF into the uterine cavity markedly reduces the pregnancy rate in monkeys (149, 150). Given these facts, small peptides that bind to the receptor without signal activation have been developed by mutation of recombinant human LIF, resulting in an inhibitor of LIFR that binds human LIFR more than 1,000 times more strongly than the native molecule (151). In vitro, this antagonist abrogated cell signaling to LIF (151). This antagonist totally blocked implantation in mice despite its short half-life, when given multiple times from day 2.5 to day 4 of pregnancy (152). To extend the half-life, the antagonist was conjugated to polyethylene glycol (PEGylated), which substantially raised serum levels of the antagonist and inhibited pregnancy when given by only 3 i.p. injections between day 2.5 and day 3.5 of pregnancy (152). The antagonist had no lethal effects on embryos (152).

Mice lacking the α-subunit of the IL-11 receptor also show an antifertility phenotype due to disturbance of the decidualization process (153). Both the IL-11 receptor mRNA and protein are highly expressed in human and monkey endometrium around the time of decidualization (154, 155). IL-11 also signals through the JAK/STAT pathway (156, 157). Similar technology to that used to generate LIFR inhibitors has been used to make inhibitors of IL-11 receptor, but these have yet to be tested in vivo.

Leptin is a peptide that regulates food intake and energy balance, but in mice with the gene encoding this protein knocked out, there is a phenotype of infertility with reduced formation of blastocysts (158). Leptin and its receptor are expressed in human endometrium during the luteal phase, and endometrial leptin secretion is regulated by the developing blastocyst (159). In cultured endometrial epithelial cells, leptin increases the levels of LIFR, LIF, IL-1, IL-1 receptor, IL-1 receptor antagonist, and phosphorylated STAT3 (160). These effects could be blocked by antibodies against LIFR or an inhibitory leptin peptide. It therefore was concluded that leptin binding to its receptor activates the JAK2/STAT3 signaling pathway, as seen for LIF (160). Leptin also inhibits decidualization of stromal cells in vitro (161). An antagonist of leptin has been made by synthesis of a small peptide comprising helix III residues 70–95 of human leptin (162). This antagonist binds specifically and with high affinity to the leptin receptor and potently inhibits leptin function in endometrial cells in vitro. An antagonist given into the uterine lumen of mice on day 3 of pregnancy substantially reduced the number of uterine horns with implantations; a scrambled peptide had no effect (163). The half-life of the antagonist was very short (~1–2 hours).

To reduce metabolism, the antagonist was PEGylated, and the half-life increased to 19–68 hours, depending on the route of administration. The antagonist, with or without PEGylation, was absorbed from a vaginal gel formulation and localized to the leptin receptors in the mouse uterus (164). Vaginal treatment with the PEGylated antagonist on days 1–6 postcoitum in mice completely prevented implantation (165). Pharmacokinetic studies with radiolabeled antagonist revealed localization mainly in the ovaries, uterus, and mammary glands, and no significant amounts in the central nervous system (164). In addition, there was no effect on energy metabolism (165). These results indicate that reproductive organs can be affected by the inhibitory peptide without any traverse of the blood-brain barrier or interference with energy metabolism.

A project supported by the WHO and the Rockefeller Foundation Initiative on Implantation identified an interesting molecule, proprotein convertase 6 (PC6), which is expressed in both mouse and human endometrium (166). PC6 is a member of a family of serine proteases responsible for posttranslational processing and activation of many regulatory proteins. Temporal and spatial mRNA and protein expression were congruent with the window of uterine receptivity (166). Inhibition of implantation was achieved by blocking PC6 protein production in the mouse uterus by use of intraluminally administered morpholino antisense oligonucleotides (167). To determine the specificity of PC6 for implantation, endometrial expression of all PCs during the menstrual cycle was studied. Only PC6 was markedly upregulated during decidualization, while the other PCs were constitutively expressed (168). Short polybasic peptide sequences have been shown to be potent inhibitors of PC6 (169), and one of these is being used in antifertility tests in mice.

Research and development key challenges

During the development of any new contraceptive drug, there are certain key questions to be considered. The most important is target validation, which can be accomplished by using knockout mice, antibodies, and known inhibitors. The target, if validated in the mouse, must have a human homolog. Specificity of expression of the target to reproductive organs is important, but it might not need to be absolute. However, other family members should not be able to substitute for the target molecule. Preferably, the target should be druggable; ion channels, enzymes, and receptors are good targets for small molecule inhibitors, but inhibitory small peptides should also be considered. Some of the antagonists under development are peptides, and this makes them unsuitable for oral administration. Other routes of administration, such as nasal sprays, transdermal delivery or vaginal application of gel formulations, and device delivery seem to be most practical. It is known that small peptides, e.g., magainin II amide (23 amino acids), delivered by vaginal tampon can be taken up in sufficient amount to prevent implantation in rhesus monkeys (170). For proof-of-principle studies in animal models, vaginal gels seem the simplest, even if less suitable for the clinic because of lower patient compliance with gels compared with pills, injectables, and devices. Assays for the specific class of molecules must be available or able to be developed. Focused libraries of compounds for the molecule class are useful. If industry is to be a collaborator, then patent protection and freedom to operate without infringing existing patents is critical. For the public sector, this might not be so important.

In the past, developers of contraceptives have tended to be overly optimistic about the development time (10, 11), which has led to donor fatigue, since in many instances donors have a much shorter time frame for results than developers. The translational process from target identification and validation to the clinic is long: if all goes well, at least 5 years, but at a small cost compared with that for clinical trials. Many leads fail at this stage and so a pipeline of potential replacements is essential for sound project management. Once the selected lead compound is in the clinic, from phase I to III can easily take 10–15 years and many millions of dollars, and so if the lead should fail, it is better that it be as early as possible in the translational process. One way to speed progress is by use of surrogate endpoints for pregnancy. For example, spermicidal compounds can be assessed by use of postcoital tests, which although difficult to do and not 100% certain, can be done in much less time and at lesser cost than a phase II efficacy trial. In contrast, an agent that prevents fertilization by inhibition of sperm enzymes will not give a positive result in a postcoital test. Ultrasound is useful for detection of follicle rupture and prevention of ovulation. Still, in many instances there is no substitute for an inhibition of pregnancy trial. How important it is to have shown a positive result in a nonhuman primate species is debatable. The difficulty here is that these studies are also complicated and expensive (large enough group sizes for statistical power) and there is, at best, a 65% conception rate in sham-treated controls. There is a lack of centers with expertise in such studies. So, all things being equal, a positive result would be helpful but should not be the gatekeeper to moving forward.

A case has been made for the need for public-private partnerships to expedite the research and development process for new contraceptives (2, 3), but in the past 10 years, the few big pharmaceutical companies active in contraceptive research have almost all closed their research laboratories and plan instead to insource new drugs when they have been fully developed by others. Given the reduction in funding for contraceptive research and development, it is important to re-energize the enthusiasm of potential funding sources, such as philanthropic foundations, as a major source for such funding. Whether it will be possible to maintain the existing low level of funding is uncertain. Inadequate funding means significant delays in the translational process. A concluding question is that if funding were not constrained, starting from where we are today, could we develop one new male and one new female nonhormonal contraceptive method by the year 2027? Despite the perils of prognostication, we are cautiously optimistic that this is possible.

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