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Mini-Review

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Update on Novel Hormonal and Nonhormonal Male Contraceptive Development

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Abbreviations: 11β-MNTDC, 11β-methyl-nortestosterone dodecylcarbonate; ADAM, A Disintegrin And Metalloproteinase; ADAMTS, ADAMs with Thrombospondin motifs; ALDH, aldehyde dehydrogenase; BET, bromodomain and extraterminal; DMAU, dimethandrolone undecanoate; EPPIN, epididymal peptidase inhibitor; HIPK4, homeodomain-interacting protein kinase 4; mAb, monoclonal antibody; MENT, 7α-methyl-19-nortestosterone; NES, Nestorone®; NICHD, National Institute of Child Health and Human Development; RA, retinoic acid; RARα, retinoic acid receptor α; RISUG, reversible inhibition of sperm under guidance; T, testosterone; TSSK, testis-specific serine/threonine kinases; TU, testosterone undecanoate.

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Abstract

Background: The advent of new methods of male contraception would increase contraceptive options for men and women and advance male contraceptive agency. Pharmaceutical R&D for male contraception has been dormant since the 1990s. The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) has supported a contraceptive development program since 1969 and supports most ongoing hormonal male contraceptive development. Nonhormonal methods are in earlier stages of development.

Content: Several hormonal male contraceptive agents have entered clinical trials. Novel single agent products being evaluated include dimethandrolone undecanoate, 11β-methyl-nortestosterone dodecylcarbonate, and 7α-methyl-19-nortestosterone. A contraceptive efficacy trial of Nestorone®/testosterone gel is underway. Potential nonhormonal methods are at preclinical stages of development. Many nonhormonal male contraceptive targets that affect sperm production, sperm function, or sperm transport have been identified.

Summary: NICHD supports development of reversible male contraceptive agents. Other organizations such as the World Health Organization, the Population Council, and the Male Contraception Initiative are pursuing male contraceptive development, but industry involvement remains limited.

Key Words: contraception, male contraception, nonhormonal contraceptive development, sperm

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Despite a variety of contraceptive options available to women, the unintended pregnancy rate in the United States remains at approximately 45% (1). Male condoms and withdrawal are the only reversible contraceptive methods available to men; however, with typical failure rates of 13% and 20%, respectively, these methods are much less reliable than available female methods like combined hormonal contraceptives (pills, rings, patches), hormonal injections or implants, hormonal intrauterine systems, and the copper intrauterine device (2). Studies across cultures, countries, and continents indicate that >50% of men would be interested in using a reversible method, if available (3), and many women would be willing to rely on their partner to use a contraceptive (4). Lack of acceptable male-controlled methods contributes to perceptions of men having limited ability to participate in reproductive decision making (5). Currently, 21% of contraceptive use in the United States involves male condom or vasectomy (6). Novel reversible male contraceptives offer the opportunity for greater reproductive agency for the male partner. Computational modeling suggests that the unintended pregnancy rate in the United States could fall by 3.5% with only 10% uptake of a male contraceptive pill among potential users and 5.3% with 15% uptake (7).

Content

Hormonal Male Contraception

Studies as early as the 1970s have demonstrated that hormonal male contraceptives can be as effective as female methods (8). Hormonal male methods build on knowledge of how hormonal methods function in women. Exogenous progestins inhibit production of gonadotropins that regulate synthesis of sex hormones (estrogen in ovaries, testosterone [T] in testes) that are needed for development of an egg or sperm. Similarities in male and female reproductive biology provide the basis for designing effective hormonal contraception for men.

High intratesticular T concentration is required for spermatogenesis. In healthy men, testicular T levels are maintained 40- to 100-fold higher than serum T levels. Below a threshold amount of testicular T, sperm production does not occur. Studies show that exogenous steroid hormone administration, an androgen alone or in combination with a progestin or a gonadotropin-releasing hormone agonist or antagonist, suppresses testicular T production through feedback inhibition of the hypothalamic–pituitary axis. However, other androgen-dependent functions such as libido, erection, ejaculation, and maintenance of muscle mass, require adequate serum androgen levels. Therefore, exogenous androgens must be added back to maintain sufficient serum levels to support those functions while keeping testicular T below the threshold to initiate sperm production.

The cutoff for normal fertility is 15 million sperm/mL; an average ejaculate contains >60 million sperm. Early efficacy studies showed that the overall pregnancy rate attributable to men with sperm concentrations ranging between 0 and 3.0 million sperm/mL was 1.4 per 100 person-years (9). Achievement of severe oligozoospermia (sperm concentration ≤1 million per mL) is associated with a pregnancy risk of approximately 2% per year (10), which is on par with highly effective female methods and the rationale for accepting 1 million/mL as the goal for sperm suppression for male hormonal methods (11). Proof-of-concept studies of combinations of progestins and T derivatives have demonstrated sperm suppression to levels that could reliably reach this goal (12-18). Sperm suppression rates of 89% to 100% have been achieved in these studies. It is unclear why some men fail to suppress. In contraceptive efficacy studies of potential male methods, confirmation of sperm suppression has been required prior to allowing men to rely on the product for contraception. It is anticipated that regulatory approval would require this confirmation; however, there is the potential for home assessment of sperm concentration with commercially available sperm assessment kits.

The search for the "male pill" has been stymied by lack of a safe, effective oral androgen, which is necessary to provide hormone addback when testicular T production and spermatogenesis are suppressed. Unfortunately, oral testosterone is cleared too rapidly to be effective as a single daily dose regimen even in combination with a progestin. Multiple doses of oral testosterone per day would be impractical for contraception. Although 17-methyltestosterone has better oral bioavailability, long-term use has been associated with hepatotoxicity. Oral testosterone undecanoate (TU) recently has been approved in the United States, but dosing is twice per day.

The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) has supported development of new androgens that bind to both androgen and progesterone receptors and potentially may serve as single-agent male contraceptives (19). Two lead candidates in development are dimethandrolone undecanoate (DMAU) and 11β-methyl-nortestosterone dodecylcarbonate (11β-MNTDC) (20, 21). The compounds are not aromatizable, which may lower serum estradiol levels if endogenous T synthesis is inhibited. Potential long-term effects on bone health are unknown, but increases in P1NP, a serum marker of bone formation, were seen in a 28-day study of oral DMAU (22). When administered orally or intramuscularly, DMAU is hydrolyzed to active drug, dimethandrolone, a derivative

of 19-nortestosterone, which binds to both androgen and progesterone receptors. DMAU has been evaluated in phase I clinical trials in the NICHD's Contraceptive Clinical Trials Network and was well tolerated (23). In a 28-day trial of 200 mg and 400 mg daily oral DMAU, serum gonadotropins were suppressed to low levels in most subjects (24). No serious adverse effects were seen. Most participants (80%) were satisfied with the method; 54% reported they would use it as their primary method of birth control if available (25). A first-in-man clinical trial of 11β-MNTDC at single doses of 200, 400, and 800 mg showed the drug was well tolerated without serious adverse effects (26). A 28-day trial of 200 mg and 400 mg daily oral 11β-MNTDC showed marked suppression of gonadotropins over the treatment period, again without serious adverse effects (27). Longer term evaluation of these progestogenic androgens is necessary to determine if they are safe and can effectively suppress sperm production.

Another synthetic progestogenic androgen, 7α-methyl-19-nortestosterone (MENT), is currently under evaluation as a possible male contraceptive (28). Initial evaluations of MENT implants to suppress sperm production were comparable to initial studies with TU, with about two-thirds of men showing dose-dependent spermatogenesis suppression (29). Improvements of the MENT implant resulting in sustained levels of MENT release are in development but require further validation.

Transdermal or injectable androgens may provide an alternative to an oral product. Testosterone gels are widely used in the United States to treat hypoandrogenism. Several trials have evaluated the ability of injectable testosterone enanthate or TU alone or in combination with a progestin to achieve sperm suppression with promising results (12-14, 16, 30). A trial combining T gel and injections of the progestin, depomedroxyprogesterone acetate, a female contraceptive product, resulted in effective sperm suppression in 90% of subjects (31). Notably, this method involved 2 Food and Drug Administration–approved products, albeit used for off-label indications.

Few regimens have been evaluated in contraceptive efficacy studies (18, 30, 32-34) (Table 1). The most recently completed contraceptive effectiveness trial built on the success of injectable TU by adding a progestin to achieve sperm suppression. This phase II multisite international clinical trial sponsored by World Health Organization and CONRAD, evaluated contraceptive efficacy and safety of separate intramuscular injections of a long-acting progestin, norethisterone enanthate, and the long-acting androgen, TU, at 8-week intervals (18). Couples (n = 320) were enrolled; 266 men suppressed to low sperm counts and the couples entered the efficacy phase. The study was terminated early per recommendation of an external safety review committee due to the frequency of reported mood changes, depression, injection site pain, and increased libido. Despite this, the combined method failure rate, including sperm nonsuppression by the end of the suppression phase, sperm rebound during the efficacy phase, and pregnancy during the efficacy phase, was 7.5%. For comparison, typical use failure rates for women using birth control pills are estimated at 7% to 9% (2). Importantly, >75% of participants said they would be willing to use the method if available.

Another regimen in development includes daily applications of a gel containing T and the progestin, Nestorone® (NES). In the proof of concept trial, use of T gel (100 mg) and NES gel (8 mg) suppressed sperm concentration to <1 million/mL or azoospermia in 89% of men compared with only 23% of men using T gel and a placebo gel (17). Suppression of serum gonadotropins (luteinizing hormone and follicle-stimulating hormone) occurred rapidly. Gonadotropin hormone concentrations that were >1 IU/L after 4 weeks of treatment predicted treatment failure (sperm concentration >1 million/mL) with 97% sensitivity (35). Most failure was due to inconsistent or nonuse of the products rather than to nonresponse to the drug regimen. When asked about acceptability of the regimen, over half of participants reported being satisfied or extremely satisfied with the method (36). A contraceptive efficacy study to evaluate combined NES/T in a single gel preparation for use as a primary method of contraception in couples is currently underway in the NICHD Contraceptive Clinical Trials Network.

Hormonal male contraceptive methods have proven effective in clinical trials. Regulatory approval of a new contraceptive drug for women usually requires 20 000 cycles of safety and contraceptive efficacy evaluation for at least 1 year of use. Long-term safety of a male method will need to be demonstrated before a drug would pass regulatory approval. Calculation of potential risk/benefit of a male contraceptive drug is challenging because men do not face medical risks associated with pregnancy and childbirth; any systemic product for men must have a strong safety profile. However, consideration of a shared risk model of the benefits of pregnancy prevention from a biologic and psychosocial context for both partners is important (37). The goal of identifying additional health benefits for male methods is especially attractive. Realistically, long-term trials in sufficient numbers of couples will require years before a product could reach the market. Regulatory agencies will need to provide guidance on what is required for approval of this new class of drugs. Additionally, pharmaceutical investment will be critical to achieve this goal.

Table 1. Male hormonal contraceptive efficacy trials

Regimen	N enrolled	N entering/ completing efficacy	Pregnancy/Failure rate per 100 couple years	Author/Sponsor
Testosterone enanthate weekly injection	271	157/119	1 0.8 (0.0 to 4.5)	WHO 1990
Testosterone enanthate weekly injection	357	268/209	4 1.4 (0.4 to 3.7)	WHO 1996
Testosterone undecanoate monthly injection	308	296/280	1 2.3 (0.5 to 4.2)	Gu et al. 2003
Testosterone undecanoate monthly injection	1045	855/733	9 1.1 (0.4 to 1.8)	Gu et al. 2009
Testosterone implant every 4-6 months + depomedroxyprogesterone acetate injection q 3 months	55	53/28	0 0 (0 to 8)	Turner et al. 2003
Testosterone undecanoate injection + norethisterone enanthate injection every 8 weeks	320	266/111[a]	4 2.2 (0.8 to 5.8)	WHO/CONRAD; Behre et al. 2016
Testosterone + Nestorone® transdermal gel applied daily	ongoing	ongoing	ongoing	NICHD

[a]Study stopped early.

Nonhormonal Male Contraception

In contrast to hormonal male contraception, where the mechanism of action is to stop sperm production through feedback inhibition of the hypothalamic–pituitary axis, the goal of nonhormonal contraceptives is to avoid the hypothalamic–pituitary axis and thereby potentially avoid side effects associated with hormones. Nonhormonal male contraceptive development is largely still in the preclinical phase and involves targeting proteins that impact either sperm production or sperm function. The number of potential targets is large and growing. Approaches to control sperm production or function vary widely; each approach will have to be evaluated to demonstrate a high degree of safety in addition to effectiveness as a contraceptive. Data from animal models suggest that such targets may prove effective if specificity is enhanced to limit off-target effects. Efforts using iterative screening, structural biology, computational modeling, and designer chemistry are being employed to move forward several potential nonhormonal male contraceptives. While measurement of sperm suppression may not provide the appropriate mechanism to evaluate effectiveness of these methods, ultimately regulatory approval will require demonstration of contraceptive effectiveness.

First studied and recognized in male rats, vitamin A (retinol) deficiency and its physiologically active metabolite, all-trans retinoic acid, have long been recognized for their role in male sterility (38). Male retinoic acid receptor α (RARα) knockout mice are infertile. The retinoic acid (RA) pathway, including conversion of retinol to retinal and finally to RA, provides opportunities for inhibitors or antagonists to stop RA synthesis and thereby stop spermatogenesis. A clinical study of a bisdichloroacetyldiamine analog in the RA pathway, WIN18,446, was used to treat over 60 men for 1 year (39). The drug was well tolerated and efficacious at inhibition of spermatogenesis. However, development of the drug was halted after finding that consumption of alcohol with the drug induced a severe disulfiram reaction, due to off-target inhibition of a liver enzyme, aldehyde dehydrogenase (ALDH)-2, which detoxifies aldehyde during alcohol metabolism. A different aldehyde dehydrogenase subfamily, ALDH1A, is involved in synthesis of RA and a testis-specific member includes ALDH1A2. Covalent and noncovalent small molecule inhibitors of ALDH1A2 recently have been developed. Ternary x-ray cocrystal structures of the inhibitors provide the structural framework for design of potent, selective inhibitors of ALDH1A2 (40).

An alternative approach in the RA synthetic pathway is inhibition of RARα. An RARα variant is essential for spermatogenesis and mouse knockouts are infertile (41). A study with the panretinoic acid receptor antagonist, BMS-189453, demonstrated reversible spermatogenesis inhibition in a mouse model (38). Structure Based Drug Design, with iterative screening, is being employed to develop potent, specific antagonists to inhibit RARα activity in the RA synthetic pathway to inhibit sperm production.

BRDT, a testis-specific bromodomain protein, is another nonhormonal target. A subfamily of bromodomain and extraterminal (BET) proteins consists of 4 members: BRD2, BRD3, BRD4, and BRDT. Testis-specific BRDT is critical for chromatin remodeling during spermatogenesis (42, 43). Male mice with homozygous BRDT null mutations are sterile (44). One study showed that JQ1, a small molecule inhibitor of BRDT, was able to cross the blood–testis barrier and cause complete, reversible contraceptive activity in male mice (45). Although effective for

contraception, JQ1 had off-target binding to other BRD proteins. Efforts are underway with different chemical scaffolds to develop optimized inhibitors of BRDT. A study entailing virtual screening, analytical testing, structure-activity relationship evaluation and compound optimization via x-ray cocrystal has resulted in different chemical scaffolds with potent BRDT inhibitory activity (46). Each BET protein has 2 bromodomain modules, and the second module (BD2) may be a target for enhancing specificity. Focused library screening and subsequent optimization has produced potent BET inhibitor candidates selective for BD2 (47).

Mechanistically different drug candidates that target Sertoli–germ cell adhesion and cause release of immature spermatids from the seminiferous epithelium have been identified. CDB-4022, an indenopyridine, inhibits mature sperm production in primates and stallions. Cessation of drug treatment results in full reversibility of sperm production with no apparent side effects (48-50). Additional drug candidates targeting Sertoli cell–germ cell interaction are indazole carboxylic acid derivatives: Gamendazole, H2-gamendazole, and Adjudin. Rats treated with oral doses of H2-gamendazole showed inhibition of fertility (51). Although the effect was reversible at low doses of the drug, at higher doses, the fertility effect was irreversible. Targeting a drug to Sertoli cells is particularly difficult due to the challenge of crossing the blood–testis barrier. To overcome this challenge, Adjudin was conjugated to a recombinant follicle-stimulating hormone–binding fragment to target the testis germ cell–Sertoli cell junction (52). However, the increase in specificity was offset by reduced oral bioavailability. Safety and reversibility of these candidates needs to be demonstrated in higher mammals to determine if they truly are candidates for development in humans.

Numerous ion channel and kinase protein targets affect sperm motility, many of which are expressed in the sperm tail region. Ions channels CatSper (a calcium ion channel) and KSper (a potassium ion channel) are sperm specific and required for male fertility (53). Animal models incorporating mutations and deletions of these genes have shown male infertility without apparent systemic effects. An in vitro study with HC-056456, an inhibitor of the calcium ion channel, demonstrated that the drug prevented hyperactivation of sperm (54). A sperm-specific potassium channel, SLO3 (also known as KCNU1) controls calcium entry through CatSper. Genetic deletion of SLO3 causes male infertility in mice (55).

Under normal function, CatSper causes sperm tail hyperactivation when progesterone binds and activates α/β hydrolase domain-containing protein 2, causing depletion of endocannabinoid 2-arachidonoylglycerol from the spermatozoa plasma membrane. Removal of 2-arachidonoylglycerol by α/β hydrolase domain–containing protein 2 releases CatSper inhibition and causes calcium influx leading to sperm activation (56). Physiologically, the cumulus–oocyte complex secretes progesterone after ovulation. Following intercourse, sperm enter the tubal isthmus through the uterotubal junction and form a reservoir (57). Sperm can remain viable in the isthmus for several days until progesterone and other triggers signal them to swim toward the tubal ampulla where fertilization may occur. A recent study demonstrated that the steroidal inhibitor, RU1968, causes dysfunction of CatSper's progesterone-mediated motility response. The inhibitor is nontoxic to human sperm and inhibits hSLO3 with approximately 15-fold lower potency than CatSper (58). It is unclear if this approach would be more appropriate for female use since it impacts progesterone function in the oviduct.

ADCY10, a major enzyme that generates cAMP in sperm, was discovered in 1999 (59). ADCY10 is the only soluble adenylyl cyclase among adenylyl cyclase family proteins of ADCY1-10. ADCY10 knockout mice have a severe defect in sperm motility and are infertile (60). Tissue analysis and subsequent tissue enrichment profile showed ADCY10 is expressed in many nonreproductive tissues (www.proteinatlas.org). More recent whole exome sequencing of 2 infertile men showed that their condition of asthenozoospermia, a condition that affects progressive motility of sperm, was due to homozygous variant upstream of nucleotide binding site of ADCY10 leading to premature termination (61). These men lead normal lives except for infertility phenotype and the potential for developing calcium oxalate kidney stones. However, the association of ADCY10 with dominant absorptive hypercalciuria has not diminished the discovery and optimization effort for ADCY10 inhibitors as new nonhormonal contraceptives (62).

ADCY10 and a sperm Na^+/H^+-exchanger form a complex critical for sperm motility (63). *Nhe8*$^{-/-}$ male mice are infertile due to disruption in acrosome formation (64). The sperm Na^+/H^+-exchanger in human sperm is mainly localized to the principal piece of the tail, and the expression pattern suggests a role in regulation of sperm motility (65). The Na^+/K^+-ATPase (sodium pump) is important in sperm motility and capacitation (66). These ion channels are found in many tissues, but the α4-subunit of the Na^+/K^+-ATPase is sperm specific and appears necessary for sperm function. The α4-subunit knockout male mice are completely infertile (67). Known inhibitors for Na^+/K^+ pumps are cardenolide analogs which have been used clinically to treat congestive heart failure. Ouabain, a cardenolide analog, has higher affinity for Na^+/K^+-ATPase α4 (ATP1A4) isoform than other somatic forms (α1, α2, and α3) in both mice and humans.

Optimization using the ouabain scaffold as a starting point may yield derivatives with specificity for the α4-subunit (68, 69). Ouabain derivatives modified at the glycone (C3) and lactone (C17) domains show picomolar inhibition for the α4 isoform with an excellent selectivity profile against α1, α2, and α3. Decrease in sperm motility in vitro and in vivo has been demonstrated for new ouabain triazole analogues (70).

Several testis-specific serine/threonine kinases (TSSKs) are important for spermatogenesis and function. In the human kinome, TSSKs belong to a 5-member testis-specific serine/threonine kinase family: TSSK1, TSSK2, TSSK3, TSSK4 (also known as TSSK5) and TSSK6. Male double TSSK1/TSSK2 knockout mice display infertility (71, 72). Stable, enzymatically active recombinant human TSSK2 protein production represents a key achievement in progress towards targeting TSSKs in humans (73). Additionally, mutation screening in 494 men with azoospermia or severe oligozoospermia compared with 357 fertile controls indicate that single nucleotide polymorphisms of the TSSK2 gene are associated with idiopathic male infertility (74). High-throughput screening of TSSK2 assays have revealed potent inhibitors (<100 nanomolar) that show promise for targeting TSSKs with small molecule inhibitors (75, 76).

The homeodomain-interacting protein kinase 4 (HIPK4) plays a role in later stages of sperm maturation and is another potential nonhormonal target (77). Studies have demonstrated that HIPK4 is expressed predominantly in round and early elongating spermatids. HIPK4 mutation in sperm display reduced oocyte binding and incompetence for in vitro fertilization (77). HIPK4 knockout mice are infertile but are otherwise healthy (78).

Sperm surface protein EPPIN (epididymal peptidase inhibitor) is another potentially druggable contraceptive target. This protein is expressed only in male reproductive tissues, testis, and epididymis (www.citdbase.org). An early study of immunization of male nonhuman primates with human EPPIN resulted in high anti-EPPIN antibody titer leading to infertility (79). Another preclinical nonhuman primate study showed that intravenous infusion of a small molecule inhibitor of EPPIN, EP055, resulted in dramatic reduction of sperm motility to ~20% of pretreatment levels. EP055 is thought to affect sperm motility by causing rapid decrease in sperm internal pH and calcium levels (80).

Another path for contraceptive discovery is via inhibition of serine protease from ejaculate, which disrupts sperm motility to reduce fertility. As such, panserine protease inhibitor, 4-(2-aminoethyl) benzenesulfonyl fluoride, inhibits semen liquefaction in vivo and drastically reduces the number of sperm in the oviduct, demonstrating inhibition of sperm transportation. Female mice treated with 4-(2-aminoethyl) benzenesulfonyl fluoride demonstrated lower fertilization rates and had significantly fewer pups per litter (81). Analysis of the semen liquefaction mechanism identified kallikrein-related peptidase 3 as another potential nonhormonal contraceptive target (82).

Numerous protein targets that affect sperm function have been identified. Several members of A Disintegrin And Metalloproteinases (ADAMs) family of proteins are expressed exclusively or predominately in testes or epididymis (83). Additionally, related members of ADAMs with Thrombospondin motifs (ADAMTS) are proposed to participate in sperm-egg adhesion (84). An ADAMTS-like protein from sea urchin is proposed to mediate species-specific sperm–egg adhesion (85). A systematic study to identify sperm membrane alloantigens in swine found more than 20 potential unique sperm membrane and 5 sperm raft proteins. Among these, ADAM1, 2, and 3 were dominant sperm membrane alloantigens (86). In ADAM3 knockout mice, sperm were unable to enter the oviduct (87). However, it is unclear if human sperm have the same requirement for ADAM3. Numerous ADAM proteins form complexes that are required for sperm–egg binding (83). Another potential candidate, Izumo1, a sperm surface protein that binds to JUNO (Izumo1R) on the egg, is required for sperm–egg fusion (88).

The absolute requirement for sexual reproduction is the process of sperm–egg recognition and subsequent fusion of the 2 gametes during fertilization. This requires fusion of gamete membranes; however, prior to this event, protein–protein recognition and interaction between egg and sperm must occur. A milestone in sperm–egg interaction was achieved when the cocrystal complex of the sperm surface protein IZUMO1 and the egg protein JUNO(IZOMO1R) was solved (88, 89). Knockdown of either protein results in infertile but otherwise healthy mice. The 2 studies provide structural insight into sperm–egg interaction at a molecular level. Although the crystal structure and other studies reveal that IZUMO1–JUNO interaction could be responsible for sperm–oocyte membrane adhesion, underlying membrane fusion steps are more complex. Studies have shown that sperm proteins, SOF1, TMEM95 (90), SPACA6 (91), and FIMP (92), are required for sperm–oocyte fusion in mice (93). Mouse knockouts of these proteins are completely infertile or severely subfertile. Discovery of proteins like CRISP2, found in male reproductive tract (94) and GLIPR1L1, an IZUMO binding protein (95), shed more light on our understanding of sperm proteins and the complexity of sperm-oocyte fusion and fertilization.

A monoclonal antibody (mAb) technology platform used against 2 sexually transmitted pathogens, HIV1 and HSV2, is being developed as an antisperm mAb (96). The antisperm antibody, H6-3C4, used in this platform was originally isolated from the blood of an infertile

woman. The antibody recognizes a carbohydrate epitope on a glycosylphosphatidylinositol-anchored glycoprotein, CD52g, found in abundance on the surface of human sperm (97). This contraceptive mAb is currently waiting for regulatory approval for a phase I clinical trial (96). A different technology upstream in the same thread but using the host to make and deliver the antibody is another attractive contraceptive model. Synthetic mRNA technology, such as recently used in the COVID-19 vaccine development effort, has been utilized to express PGT121, a well-established HIV-neutralizing antibody. In the HIV antibody work, inoculation of the female sheep reproductive tract with synthetic mRNA results in high levels of antibody expression (98). This new paradigm of antibody delivery to the female reproductive tract causing significant antibody production may be applied to contraceptive development. This may be facilitated by the recent synthesis of mRNA encoding antisperm mAb (96).

Gossypol is a polyphenolic aldehyde–containing compound isolated from the cotton plant (99, 100). Gossypol was implicated in causing infertility in the 1950s and 1960s when peasants in rural areas of China began to press uncooked cotton seeds for cooking oil. Women and men who consumed the raw untreated oil became infertile. Subsequently, a gossypol-free diet resulted in eventual recovery for women. However, some men did not recover from their infertility and impotency. This indicated that quantity and duration of cotton oil consumption impacted the rate of recovery, likelihood of permanent infertility, and led to the idea that gossypol could be used as a male contraceptive (99). Clinical studies of gossypol for male contraception were initiated in China in the 1970s and 1980s. In total, more than 8000 volunteers participated in these studies. Gossypol was highly efficacious as a male contraceptive; however, the narrow therapeutic window and frequent association with hypokalemia and irreversible sterility caused termination of further clinical development (101, 102). A more recent animal study in ewes investigating consumption of a gossypol-rich diet during the critical period of fetal development and early neonatal life in offspring showed significant arrest of growth and testis weight. Reduced testosterone levels and a significantly altered testis transcriptome were seen in male offspring. Many of these altered testis transcriptomes are implicated in testis development and sperm biology (103).

Extract from *Trypterigium wilfordii* Hook. f., commonly called Thunder God Vine, has been used in Chinese herbal medicine for many years. For more than 50 years, the refined extract was used to treat rheumatoid arthritis, chronic nephritis, chronic hepatitis, and various skin disorders (104, 105). Triptolide, a major component of the extract, belongs to the class of chemicals called diterpene epoxides (106). Indication of potential contraceptive potential for triptolide was discovered when rheumatoid arthritis patients treated with the extract developed necrospermia or azoospermia (105, 107, 108). Subsequent studies in rats showed that *T. wilfordii* extracts containing diterpene epoxides cause severe decrease in epididymal sperm count and motility in male rats (104, 105, 108-112). Similar to gossypol, prolonged exposure was associated with irreversible infertility in rodents (110, 112). Unfortunately, triptolide's immunosuppressive properties likely would prevent it from being developed as a contraceptive.

A commonly used plant in Jamu preparation, an Indonesian traditional medicine, *Justicia gendarussa* has been used as a male contraceptive in Papua. Additionally, this plant is used to treat a variety of ailments including arthritis, cancer, as an anti-inflammatory, antibacterial, and antifungal. In particular, gendarusin A and B, flavonoid scaffold analogs, are thought to be active metabolites responsible for eliciting the contraceptive effect, possibly by decreasing human sperm hyaluronidase activity (113). An unpublished clinical trial performed in Indonesia reported contraceptive efficacy of extract of the *J. gendarussa* plant if ingested by the male partner daily for at least 20 days before having intercourse during the female's ovulatory period (114). Fertility was restored within 30 days after last usage and minimal side effects were reported. Additional study on its mechanism of action and evaluation of longer duration of use would be required prior to regulatory approval.

Focusing on a local versus systemic approach, development of a nonhormonal method to reversibly block the vas deferens began in the 1970s in India. The procedure, called RISUG (reversible inhibition of sperm under guidance), involves injection of the polymer styrene maleic anhydride mixed with the solvent dimethyl sulfoxide into the vas deferens (115). The polymer is thought to damage sperm, making them ineffective. The procedure was used in the first human in 1989. By 2000, RISUG was evaluated in a phase III clinical trial in India with promising results. However, an inspection of the Indian facilities by World Health Organization raised concerns that studies were not done according to international standards, and further development was curtailed. Intellectual property rights to RISUG were acquired by the Parsemus Foundation, a nongovernmental organization, in 2010, who then developed Vasalgel™, also a styrene maleic anhydride acid polymer dissolved in dimethyl sulfoxide. Vasalgel™ is purported to act as a mechanical barrier to sperm passage. It is thought that sperm flow can be restored by flushing the vas with an injection of sodium bicarbonate solution. Studies in rabbits and monkeys have been completed (116, 117). Similar technology was used in China in the mid-1980s,

in which a polyurethane elastomer plug was injected into the vas deferens to achieve azoospermia in 96% of men, but these results were seen 24 months after injection (118, 119). Reversibility and return to fertility was demonstrated through surgical removal of the plug (120). A small company, Contraline, is developing a hydrogel, ADAM™, for injection into the vas deferens to block the flow of sperm but not other fluids (121). The gel ideally could be dissolved to restore fertility.

The Parsemus Foundation also has supported development of the "clean sheets" pill that allows for orgasm without ejaculation. They developed initial drug prototypes based on side effect profiles of 2 therapeutic drugs, thioridazine and phenoxybenzamine, which inhibit semen emission without affecting erection or orgasm. Further optimization of the prototypes is on hold pending a funding source.

A contraceptive infertility target database was established in 2018 as a tool to identify male and female reproductive tissue specific transcriptome and proteome targets. This database, Contraceptive Infertility Target Data Base (CITDBase: https://www.citdbase.org), is a curation of publicly available transcriptomic, proteomic, and immunohistochemistry (antibody-Ab) data from human tissues. Filters are applied for adjusting the degree of separation between reproductive and nonreproductive tissues in mining of gene/protein targets. This website allows investigators to mine transcriptomic and proteomic resources to identify high quality contraceptive/infertility targets.

Conclusion

Although some nonhormonal inhibitors and natural products have been evaluated in humans, nonhormonal male contraceptives are in early stages of development. In addition to protein targets and small molecule inhibitors described above, active research is ongoing for nonhormonal contraceptive target discovery and validation. Numerous laboratories are engaged in discovery and optimization of small molecule inhibitors. It is hoped that some of these small molecule contraceptive agents would enter preclinical development and advance into clinical development.

Introduction of an effective reversible male contraceptive method has potential to substantially reduce unplanned pregnancy rates. It likely would represent a new market opportunity rather than creating a significant reduction in the use of existing female contraceptive methods and would provide an opportunity for men to better engage in reproductive decision making. How a possible risk to 1 individual may be mitigated by prevention of potential health consequences in another individual provides an interesting regulatory consideration for evaluation of systemic male contraceptive agents. At the current pace of drug development, regulatory approval for a new male product in the United States likely would not occur until at least 2030. This timeline potentially could be shortened with increased resources and investment into the development pipeline.

Additional Information

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