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Male Contraception: Past, Present and Future

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Abstract: Current contraceptive options available to men include withdrawal, condoms, and vasectomy, each of which has its own drawbacks. In this chapter we will describe the pros and cons for each, as well as methodological and product updates. Statistics from the U.S. Centers for Disease Control on acceptance and satisfaction will be included. Advances in vasectomy and reversal will be presented. Methods to develop new contraceptive technologies fall into two categories: hormonal and non-hormonal. Many targets and strategies have been proposed for non-hormonal male contraception within the testis. Targets include structural components in the testis, as well as enzymes, ion channels and other proteins specific to spermatozoa. Here we provide an overview of the spermatogenic mechanisms and proteins that have received research interest to date. We also discuss potential novel targets, such as ubiquitin specific proteases, that warrant greater research emphasis.

Keywords: Hormonal, male contraception, sperm, targets, testis.

1. INTRODUCTION

As was pointed out in a recent review [1], there are two main obstacles to research on a male contraceptive technology: The first is the lack of interest by "Big Pharma" in going forward with research or research funding in the development of a useful product. The second is that women might not embrace the technology because they have the most to lose, *i.e.* an unwanted pregnancy. There's not much that can be done to increase the interest of the pharmaceutical industry except the completion of basic research showing an immediately proven, reliable product. It's always possible that a forward-looking Pharma executive could decide to embark on product development, but unlikely. A stable relationship is the strongest reason for women to accept a man's willingness to be the contraceptor. More urgently, some women suffer unwelcome side effects from hormonal contraception and therefore are more amenable to the partner being the responsible party.

Current contraceptive options available to men include withdrawal, condoms, and vasectomy, each of which has its own drawbacks. In this chapter we will describe the pros and cons, as well as methodological and product updates for each. Statistics from CDC on acceptance and satisfaction will be included. Advances in vasectomy and reversal will be presented.

Methods in development of a contraceptive technology fall into two categories: hormonal and non-hormonal. Development of a male hormonal contraceptive has been pursued for many years as the most approachable method that would be successful. The challenges to this approach include: the need for an injectable formulation, unacceptable side effects, and failure to adequately suppress spermatogenesis in 5-10% of men. Hormonal treatment that suppresses gonadotropins is associated with, but does not ensure, adequate suppression of spermatogenesis. There is a clinical trial underway involving combined delivery of testosterone (T) and nestorone (a nonandrogenic progestin) by transdermal gels for the suppression of spermatogenesis. Transdermal gels are a more acceptable method of delivery, and efficacy across diverse ethnic groups was achieved with 88–89% of treated men achieving sperm concentrations below 1 million/ml [2]. This information may be utilized to allow for rapid identification of non-responders in male hormonal contraceptive trials.

2. OVERVIEW OF CURRENT NON-HORMONAL MALE CONTRACEPTIVE METHODS

In the United States, only two methods of non-hormonal contraception are currently marketed for male use: condoms and vasectomy. Of these two methods, only condom use is reliably reversible.

Condoms: This approach relies upon an exterior barrier method and has been used for contraception for at least 400 years [3]. Latex condoms, the most common type, help prevent pregnancy, and HIV and other STDs, as do the newer synthetic condoms. "Natural" or "lambskin" condoms also help prevent pregnancy, but may not provide protection against STDs, including HIV. Typical use failure rate: 18%. Condoms can only be used once. Condoms, *K-Y*® Jelly, or water-based lubricants are readily available for purchase at a drug store. Latex condoms should not be used with oil-based lubricants such as massage oils, baby oil, lotions, or petroleum jelly, which will weaken the condom, causing it to tear or break. Condom use is highly recommended for disease prevention; there are a number of condom distribution programs worldwide that focus more on this aspect. The effectiveness of Family Planning Methods in general

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has been detailed most recently [4], and serves to emphasize the need for additional contraceptive technology.

Another "barrier" method, involving a number of materials and procedures developed using clinical trials in countries outside the U.S., blocks the vas deferens as an alternative to vasectomy. This method includes injectable biomaterials that form a polymer when mixed, or the use of a silicone implant. China and India appear to be active in developing this method. RISUG (which is an acronym for "Reversible Inhibition of Sperm Under Guidance") is similar to vasectomy but with several advantages, the most significant being that it may be more readily reversible (http://www.newmalecontraception.org/risug/) [5]. Researchers achieve this feature by injecting a polymer (a gel) into the vas deferens. To restore fertility, whether after months or years, the polymer is flushed out of the vas with another injection. RISUG is composed of powdered styrene maleic anhydride (SMA) combined with dimethyl sulfoxide (DMSO). The resulting gel is injected into the vas deferens. However, RISUG reportedly does not rely on completely blocking the vas lumen for its effectiveness. The vas is a notoriously difficult tube to block completely, since it will often stretch around a solid plug and begin to leak — or if the plug is big enough that the vas can't stretch any farther, the vas may rupture. But apparently RISUG is not just an inert plug. The RISUG material is thought to also actively kill any sperm that come into contact with it. Aside from hormonal regimens, RISUG is the only new male contraceptive advanced to Phase III clinical trials (http://www.newmalecontraception.org/risug/).

Vasectomy: During the 2014 American Society of Andrology Meeting in Atlanta, Georgia [6], Jay Sandlow, M.D. presented a lecture entitled "Current Standards and Controversies Regarding Vasectomy and Vasectomy Reversal". According to Sandlow, "Vasectomy is a safe and effective method of permanent contraception. In the United States, it is employed by nearly 11% of all married couples and performed on approximately half a million men per year, which is more than any other urologic surgical procedure". Note that this is far less than the number of female sterilizations by tubal ligation in the United States and worldwide, even though vasectomy is less expensive and associated with less morbidity and mortality than tubal ligation [6]. This author also notes that vasectomy reversal is much less common than vasectomy with approximately 4-6% of men ultimately requesting a reversal. Presumably such a request would involve a change in lifestyle due to loss of a partner by divorce or death, or a renewed desire to sire offspring.

Research by the Centers for Disease Control (CDC) has estimated there is a probability of 11 failures per 1,000 procedures over 2 years; half of the failures occurred in the first three months after the vasectomy, and no failures occurred after 72 weeks [7]. CDC research also examined regret among women whose partner underwent a vasectomy [8]. In interviews with female partners of men who received vasectomies, CDC found that while most women did not regret their husband's vasectomies, the probability of regret over 5 years was about 6%. The American Urological Society did an exhaustive literature search that led to development of guidelines on the surgery (http://www.auanet.org/content/media/vasectomy.pdf) [9]. Sandlow (2014) summarized these results [6], pointing out that "The document reviews the entire procedure from counseling to follow up, including best practice for performing the procedure, complications and future areas for research". Literature regarding vasectomy reversal outcomes has demonstrated an overall high success rate for reversal, dependent upon various factors, including time from vasectomy, surgeon training and experience, and most importantly, female partner factors. Multiple studies have reported on cost-effectiveness in comparison to IVF, with most showing lower costs and similar outcomes for reversals. Other studies have examined the role of vasectomy reversal for post vasectomy pain, with good efficacy in "carefully chosen patients". These Guidelines address many of the controversies about this minimally invasive and highly effective form of permanent male contraception.

Also described on the Internet is the *No Scalpel Vasectomy* (http://www.noscalpelvasectomy.com) [10], a technique used to perform the vasectomy using one single puncture and a local anesthetic. The puncture is made in the scrotum and requires no suturing or stitches. The actual interruption of the vas, which is done using the *No Scalpel Vasectomy* technique, is identical to the interruption performed using conventional techniques. The *No Scalpel Vasectomy* technique is simply a more elegant and less traumatic way for the surgeon to control the vas and proceed with its interruption, at least according to the practitioners. The primary difference compared to conventional vasectomy is that the vas deferens is controlled and grasped by the surgeon in a less traumatic manner. This results in less pain and fewer postoperative complications.

Non-hormonal methods that haven't yet reached the clinic, but which are active areas of scientific research, involve searching for targets in testes or spermatozoa that are specific and reversible. Targets include structural components in the testis, as well as enzymes, ion channels and other proteins specific to spermatozoa. It is essential that a biological basis be established for validating a target. Targeted disruption of a gene, or identification of a gene mutation that shows an infertility phenotype, has identified several candidates. The next steps, which involve screening libraries of potential inhibitors, require collaborations between medicinal chemists and reproductive biologists to demonstrate feasibility of target disruption and reversible inhibition of fertility. This is time-consuming, expensive and considered risky by study sections. Funding is limited and progress is slow. Since contraception doesn't involve a life-threatening disease, it is a challenge for this research to attract the resources necessary for success in developing a male- specific product. The fact that human overpopulation in many countries threatens the quality of life on earth is not viewed as something sufficiently urgent for research dollars.

3. NON-HORMONAL TESTIS TARGETS AS ACTIVE AREAS OF RESEARCH

Numerous targets and strategies have been proposed for non-hormonal male contraception within the testis [3, 11-14]. The ultimate goal is to interfere with spermato-

genesis. While a strength of this approach is the possibility of fewer side effects than hormonal-based contraception, many obstacles have been encountered including toxicity, lack of efficacy and consistent reversibility, inappropriate delivery methods, and expense. Additionally, most pharmaceutical industry research into non-hormonal targets stopped completely by 2009 [15]. Here we provide an overview of the spermatogenic mechanisms and proteins that have received research interest to date, summarized in Fig. (**1**). We also discuss potential novel targets that warrant greater research emphasis.

3.1. Spermatogonial Differentiation and Meiotic Entry

3.1.1. TEX14

The division of most spermatogonia results in the formation of intercellular bridges, connecting male germ cells as syncytia. The germ cell-specific protein Testis expressed gene 14 (TEX14) converts cellular midbodies into stable intercellular bridges [16]. In the absence of *Tex14*, mouse spermatogonia continue to generate midbodies in telophase of mitosis. However, no intercellular bridges form under these conditions and spermatogenesis collapses prior to meiosis [17]. TEX14 harbors three amino-terminal ankyrin repeats, a central dead-kinase domain, and coiled-coil motifs [18]. Targeting this protein and the mechanisms by which it functions in spermatogonia for male contraception is an innovative strategy, but one that requires further basic research.

3.1.2. BDADs (WIN 18,446)

Vitamin A metabolism has long been considered as a target for male contraception [19]. Spermatogonial differentiation and meiotic entry requires retinoic acid, a principal metabolite of vitamin A [20, 21]. Male rats and mice deficient in vitamin A, as well as retinoic acid receptor (RAR) knockout mice, are infertile [20, 22]. Bisdichloroacetyldiamines (BDADs) are compounds that were initially synthesized in the late 1950s and early 1960s. WIN 18,446 is one such BDAD that was shown in 1961 to reversibly inhibit spermatogenesis in humans upon oral administration [23, 24]. However, severe side effects occur when this compound is mixed with alcohol [14]. The mechanism of action was recently identified as the inhibition of aldehyde dehydrogenase 1a2 (ALDH1a2), required to convert vitamin A to retinoic acid [23]. Thus, development of a WIN 18,446 derivative that is specific for the testis aldehyde dehydrogenase is a promising approach to a non-steroidal male contraceptive that could be taken orally.

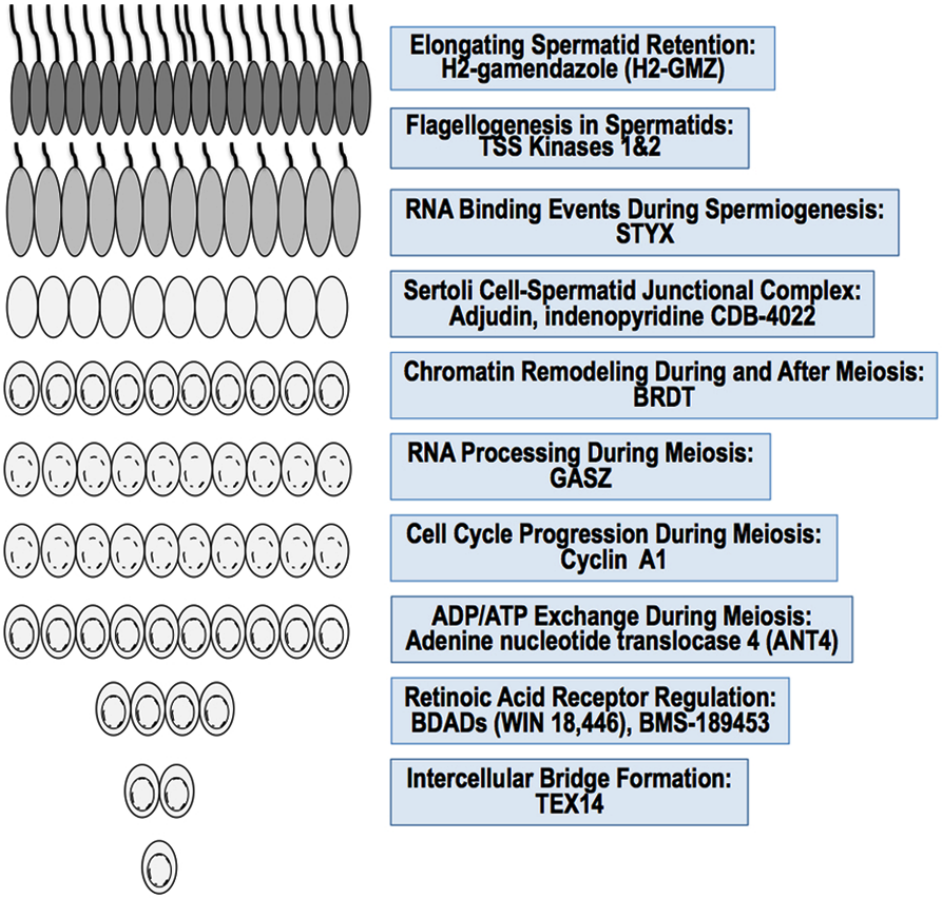

Fig. (1). Non-hormonal targets at the different stages of spermatogenesis in rodents. Schematic diagram summarizing the progression of male germ cell development from a spermatogonial stem cell (bottom) to fully elongated spermatids (top) and the various proteins (or chemical compounds) at the different biological stages. Molecular events during spermatogenesis and their corresponding validated targets are labeled in the grey shaded boxes.

3.2. Meiotic Progression

3.2.1. ANT4

As meiosis proceeds, the number of mitochondria within developing spermatocytes increases substantially. This expansion suggests a critical need for ATP production and usage. The translocation of ADP and ATP across the inner mitochondrial membrane is required for ATP utilization and is mediated by Adenine nucleotide translocases (ANTs). ANT4, known also as SLC25A31 and AAC4, is testis-specific and essential for the transition of leptotene spermatocytes into pachytene and diplotene spermatocytes [25]. The generation of male mice lacking *Ant4* results in meiotic arrest. As such, the screening of small molecules that can selectively inhibit ANT4 is a worthwhile strategy that is currently underway.

3.2.2. Cyclin A1

Cyclin A1, encoded by *Ccna1*, is specific to germ cells in mice and is present at high levels in the testis in humans [26]. Male mice lacking *Ccna1* are sterile resulting from a block in spermatogenesis before reaching the first meiotic division [27]. Meiotic arrest reveals increased germ cell apoptosis, decreased Cdc2 kinase activation at the end of meiotic prophase, and abnormal desynapsis [27]. Thus, Cyclin A1 is critical for spermatocytes to transition through the first meiotic division. This function cannot be compensated by other cyclin family members.

3.2.3. GASZ

Identification of a Germ cell-specific protein with four Ankyrin repeats, a Sterile alpha motif, and a basic leucine Zipper domain (GASZ) that is well conserved across species revealed its localization to the chromatoid body, a cellular site where RNA processing occurs [28-30]. Such events include translational repression, RNA interference, and mRNA degradation. Loss of GASZ in mice inhibits the development of zygotene spermatocytes to the pachytene stage and induces male sterility [31]. Increased hypomethylation and expression of retrotransposons occurs in such mutants, with a downregulation of piRNAs [31].

3.3. Meiotic Exit and Spermiogenesis

3.3.1. BRDT

BRDT is a bromodomain protein member of the BET family, binding acetylated histone 4 associated with meiotic and post-meiotic chromosomes in sperm [32, 33]. Although named as testis-specific, it is also found in ooctyes [34]. Male mice homozygous for mutations in the first bromodomain region of BRDT are infertile, but otherwise appear normal indicating the specificity of this protein [35]. Female mice appear normal and are fertile. Small molecule JQ1, a thieno-triazolo-1,4-diazepine designed as a prototype for binding to the bromodomain in the BET protein family, is highly selective for this specific bromodomain [36]. Matzuk and colleagues have recently published work on the use of JQ1 as an i.p. daily injection, male contraceptive in mouse [37]. Complete and reversible contraception was achieved in a small group of male mice and histochemical analysis indicated that effects were at the level of spermatocyte to spermatid maturation with a concomitant decrease in testicular volume. Off target effects were not observed and circulating levels of FSH, LH and testosterone were normal. Effects on offspring sired by withdrawn males were not observed. Enthusiasm for this approach is tempered, however, since the severe decrease in testicular size (40-70%) would likely be unacceptable to many men [38].

3.3.2. BMS-189453

Recently, a retinoic acid receptor antagonist (BMS-189453) that is able to bind to all three RARs (α,β,γ) has been studied for contraception in male mice [39]. It causes a failure of spermatid alignment and sperm release. It is extremely effective, highly reversible, and shows low off-target effects. This research is at the stage of developing an RARα-specific antagonist [14, 15].

3.3.3. Adjudin, H2-GMZ, CDB-4022

Adjudin is a derivative of the chemotherapuetic compound lonidamine [14, 40, 41]. It disrupts the bridges between spermatids and Sertoli cells thus disrupting sperm maturation. Although highly effective and reversible in rodents, there are off-target effects in other organs such as liver that have essentially halted investigations of the use of this formulation. Conjugation with a portion of the FSHß subunit allows more specific targeting to the testis, but increases cost. Delivery (injection) is less than optimal. H2-gamendazole (H2-GMZ) is another lonidamine derivative that has been investigated as a non-steroidal male contraceptive with some promise, but significant concerns about toxicity and reversibility, in rodent [14]. CDB-4022 is a chemically distinct compound, an indenopyridine, which also works, at least in part in the seminiferous epithelium to disrupt germ cell adhesion [42]. Reversibility is species dependent - high in primate models, but low in rats [41].

3.3.4. STYX

STYX is a pS/T or pY interaction protein that contains protein tyrosine phosphatase motifs [43]. It is catalytically inactivated by the substitution of the active-site cysteine with glycine. Deletion of the gene encoding STYX disrupts the development of round and elongating spermatids in mice [44]. Sperm production is decreased by more than 1,000-fold and abnormal sperm head formation renders the males infertile [44].

3.3.5. TSS Kinases 1&2

Testis-specific serine threonine kinases 1 and 2 (TSSK 1&2) are restricted to elongating spermatids and are required for flagellogenesis [45, 46]. In the absence of TSSK 1&2, spermiogenesis is arrested [47]. Substrate TSKS localizes to spermatid centrosomes in humans and mice during flagellogenesis and persists in mature sperm centrioles [46]. Current focus is on determining the three dimensional structures of TSSK 1&2 and TSKS, and identifying small molecules that bind to and inhibit the kinases.

3.4. Potential Novel Targets

3.4.1. DUB Family of USPs

Ubiquitin specific proteases (USPs) are a subclass of deubiquitinating (DUB) cysteine proteases. Of the approximately 95 DUBs in the human genome, at least 58 are USPs [48]. To date, 3 USPs have been localized to male germ cells (USP2, USP14, USP26), and at least 5 other family members have been detected at high levels in the testis (USP25, USP31, USP42, USP44, USP9y) [49-57]. USP2, with predominant isoforms USP2a and USP2b, is highly expressed in the testis [49]. In rats, USP2a and USP2b localization is restricted to late (step 16-19) elongating spermatids, distributing in the nucleus, residual bodies, and other extranuclear regions [49]. Gene-targeted *Usp2*$^{-/-}$ male mice exhibit subfertility, generating 12% as many offspring as littermate controls [58]. Testis weights and sperm counts are normal, and spermatogenesis is largely unaffected, with the exception of abnormal aggregations of elongating spermatids and multinucleated cells forming in some seminiferous tubules. *Usp2*$^{-/-}$ epididymal sperm are morphologically normal, are capable of undergoing the acrosome reaction, and exhibit normal motility in culture media, but are immotile when placed in PBS lacking nutrients [58]. *In vitro* fertilization with *Usp2*$^{-/-}$ sperm results in a 10% fertilization rate compared to >60% fertilization using control sperm. Zona pellucida removal and intracytoplasmic sperm injection both restore fertilization rates to control levels [58]. Collectively, these results suggest that *Usp2*$^{-/-}$ sperm have a defect in their ability to generate chemical energy, a process essential for nutrient-poor conditions and for penetrating the zona pellucida. USP14, meanwhile, distributes in the cytoplasm of round and elongating mouse spermatids and associates with the postacrosomal segment of spermatid nuclei in steps 14-16 [51]. In step 16 elongating spermatids, USP14 localizes to the redundant nuclear envelope at the base of the spermatid nucleus. Male mice containing genetic mutations in *Usp14* are infertile, exhibiting reduced numbers of germ cells, impaired spermatid elongation, and in some cases, hyperproliferation of Leydig cells [51]. Testis weights are reduced by 50% and sperm counts are diminished 100-fold, with abnormal sperm residing in mutant epididymides. The third reported germ cell-associated USP family member, USP26, is linked to infertile men exhibiting asthenozoospermia, oligozoospermia, and azoospermia, although this association is controversial [59, 60]. Intense USP26 immunostaining occurs in the cytoplasm of elongating mouse spermatids (steps 9-16), as well as in Leydig cells and the corpus, caput, and cauda regions of the epididymis [52]. No gene-targeted or mutant mice have been generated. As a result, *Usp26* loss-of-function effects are unknown. Characterization of these three USPs in the testis raises the possibility that other family members exclusively localize to elongating spermatids and mature sperm, and that they might serve as novel contraceptive targets.

Any non-hormonal contraceptive will need to be reversible in order to appeal to the largest cross-section of men that will make this a marketable product. Although several of the compounds described above show reversibility in studies using small numbers of animals, these studies were all done over a relatively small percentage of the reproductive life span of the test animal. In human populations, these drugs would potentially be used for years before the subject would want to regain fertility. Compounds (and proposed targets) that function early in the spermatogenesis pathway can, or may be expected to, cause significant tissue remodeling in the testis. The long-term consequences of such remodeling must be of concern. Moreover, compounds directed at post-meiotic targets must be able to cross the blood-testis barrier [61]. The method of delivery is another important issue. Daily injections are not optimal and less frequent injections will only be acceptable to a sub-population of men. Oral or perhaps patch methods are necessary.

CONCLUSION

In this article we have referred to the current outcome of family planning measures and presented the most common male-specific methods of non-hormonal contraception. We have also tried to emphasize the need for additional, user-friendly methods with a description of ongoing research in this area. Clearly, the testis with its myriad of cell types and stages presents a challenging target. Attacking this target requires caution to ensure that any chemical insults are reversible and site-specific. As described previously [1], developing male gametes and mature spermatozoa contain a number of cell specific proteins required for function. Disrupting this function with drug selectivity is the goal of several research projects. The probability of reversibility is high making sperm attractive targets. This research is too early in its development to assess success and it's likely that this is a consequence of underfunding. In order to achieve progress, we suggest that the consequences of overpopulation in the world and its effect on the human quality of life make contraceptive development a high priority item for additional research support.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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