# New frontiers in nonhormonal male contraception

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## Abstract

The world's population is nearing 6.8 billion, and we are in need of a male contraceptive that is safe, effective, reversible and affordable. Hormonal approaches, which employ different formulations of testosterone administered in combination with other hormones, have shown considerable promise in clinical trials, and they are currently at the forefront of research and development. However, the long-term effects of using hormones throughout a male's reproductive life for contraception are unknown, and it may take decades before this information becomes available. Because of this, many investigators are aiming to bring a nonhormonal male contraceptive to the consumer market. Indeed, there are several distinct but feasible avenues in which fertility can be regulated without affecting the hypothalamus-pituitary-testis axis. In this review, we discuss several approaches for fertility control involving the testis that one day may lead to the development of a nonhormonal male contraceptive.

## 1. Introduction

At the writing of this review, the world's population is estimated to be 6.8 billion and rising rapidly. Still, safe, effective, reversible and affordable contraception continues to be an unmet need for many couples. While most women commonly assume the responsibility of family planning, surveys have shown that men in general are interested in playing a greater role [1,2]. This is probably because none of the currently available contraceptive options for men (i.e., condom, vasectomy, withdrawal or periodic abstinence) are ideal. For instance, there are concerns that vasectomy associates with an increased incidence of epididymitis or orchitis resulting from the development of anti-sperm antibodies [3–5]. As for the condom, which has been used for at least 400 years, the failure rate is unacceptably high at 10–18% with typical use [6]. Thus, studies that aim to identify new and better solutions for fertility control in males are a priority.

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Presently, there are several male contraceptive approaches being investigated, including delivery of hormones which affect sperm production, partially blocking the vas deferens to prevent the passage of sperm, immunocontraceptives and administration of different compounds that can arrest spermatogenesis, thereby leading to infertility. Of these, hormonal contraceptive methods have gained the most ground since many regimens (e.g., testosterone enanthate, testosterone decanoate, testosterone undecanoate) have been tested in clinical trials and shown to be effective [7–9]. The premise for hormonal male contraception is that exogenous administration of different testosterone formulations in combination with other hormones (e.g., etonogestrel implants or depot medroxyprogesterone acetate injections) can suppress the secretion of pituitary luteinizing hormone (LH) and follicle stimulating hormone (FSH), resulting in azoospermia in many, but not all, men. While hormonal contraceptives appear to be safe, additional studies are needed to investigate the long-term effects of testosterone administration not only on androgen-dependent organs such as the prostate but also on overall male health since contraceptives would be used by healthy individuals for extended periods of time.

Besides hormonal male contraceptives, there is also an interest to develop nonhormonal contraceptives and several different approaches are under investigation. For example, studies from our own laboratory on adjudin have shown this compound to be effective in inducing germ cell loss from the seminiferous epithelium in rats and rabbits by specifically affecting germ cell adhesion in the testis but not in other organs, resulting in transient infertility [10–14]. Other studies employing transcriptional profiling of genes in the testis aim to identify novel targets which may halt sperm production, development or function. Thus, the need for non-hormonal male contraceptives is also being addressed by several different lines of research. Herein, we focus on the current status of nonhormonal male contraceptive research and development.

## 2. Prospects for nonhormonal male contraception

### 2.1. Reversible inhibition of sperm under guidance

Reversible inhibition of sperm under guidance (RISUG) involves injection of steric maleic anhydride (SMA) combined with dimethyl sulfoxide (DMSO) into the vas deferens to create a partial obstruction, and as sperm passes, its plasma and acrosomal membranes, midpieces and tails are severely destroyed, resulting in infertility [15,16]. RISUG may be a promising alternative to vasectomy since infertility was shown to be reversed after short-term vas occlusion in langur monkeys and rats [16–18]. A recent report also describes a novel formulation defined as smart RISUG that was synthesized by dispersing iron oxide and copper particles into SMA-DMSO, thus allowing detection of the implant by X-ray and magnetic imaging [19]. Interestingly, magnetic particles have been shown to agglomerate and to bind proteins [20], whereas copper particles have been demonstrated to displace molecules on the surface of sperm [21]. This formulation also appears to provide improved spermicidal action over the original formulation of RISUG [19]. The safety and efficacy of RISUG in humans has been previously shown [22,23], and RISUG is presently undergoing Phase III clinical trials in India [24]. However, additional studies are needed on RISUG.

## 2.2. Contraceptive vaccines

Immunocontraception involves the use of antigens/antibodies to target different aspects of gamete production and function as a means of inducing infertility [25–29]. For example, vaccines based on luteinizing hormone-releasing hormone/gonadotropin releasing hormone (GnRH) have been tested, and they have been shown to affect testosterone production, resulting in a decrease in testis and prostate weights [30,31]. Immunocontraception that targets different sperm antigens to affect fertilization and fertility has also been investigated. For instance, immunization of male monkeys with human recombinant epididymal protease inhibitor (EPPIN), a serine protease inhibitor expressed by the testis and epididymis [32–34], altered sperm progressive motility (as the ability of anti-EPPIN-treated spermatozoa to utilize cAMP was compromised [35]) and resulted in reversible infertility [36,37]. Additional studies reported that EPPIN binds to semenogelin I [38], a protein in the coagulum that renders sperm immobile [39], and anti-EPPIN antibodies appear to affect the interaction between EPPIN and semenogelin [40]. However, after nearly 3 decades of research, there are no immunocontraceptives on the market for human use. This is because contraceptive vaccines have been shown to be ineffective in inducing long-term infertility in all subjects which is probably due to differences in host immune responses. There are other obstacles as well. The entire spermatozoon cannot be used for the development of a vaccine because it contains several antigens that are also likely to be expressed by other cells in the body, that is, the antigen must be sperm-specific. Moreover, any antigen that is to be used for immunocontraception has to be present on the cell surface [41]. Equally important, the antigen has to be able to raise high-titer antibodies following administration and to interrupt fertility. Faced with these challenges, many investigators are currently re-examining the feasibility of using immunocontraception for fertility control. For instance, genetically engineered antibodies (e.g., multi-epitope vaccines and single chain variable fragment antibodies) that are likely to enhance efficacy may hold promise in the future [42].

## 2.3. $Ca^{++}$ channel blockers

$Ca^{++}$ is critical for sperm motility, capacitation and the acrosome reaction [43] and many $Ca^{++}$-permeable channels have been identified in sperm including cyclic nucleotide-gated channels, voltage-gated channels and transient receptor potential channels [44–46]. In addition to these $Ca^{++}$ channels, sperm expresses unique $Ca^{++}$ channels, CAT-SPERS, which localize to the principal piece [47–49]. All four *CatSper* genes are indispensable for male fertility as a disruption in any one of these genes failed to initiate hyperactivation, resulting in sterility [48–52]. Indeed, HC-056456, a CATSPER channel blocker, was recently shown to prevent hyperactivated motility in an in vitro study [53]. As such, targeting CATSPER function may be a promising approach for male contraceptive development since effects would very likely be restricted to the testis. Along similar lines, nifedipine is a widely prescribed anti-hypertension drug that blocks $Ca^{++}$ influx into sperm and affects sperm membrane cholesterol, thereby compromising fertility [54]. The $Ca^{++}$ blockers verapamil and diltiazem are also known to affect sperm motility in rats [55]. While calcium blockers such as nifedipine which affect male fertility have passed extensive safety tests in humans, the use of anti-hypertension drugs for the control of male fertility does not

appear to be an appealing contraceptive approach. At present, additional research is needed to identify drugs that would block CATSPER but not other ion channel proteins.

## 2.4. Indenopyridines

CDB-4022 [4aRS,5SR,9bRS]-2-ethyl-2,3,4,4a,5,9b-hex-ahydro-8-iodo-7-methyl-5-[4-carbomethoxyphenyl]-1H-indeno[1,2-c]pyridine-hydrochloride], an indenopyridine, is a compound that was initially developed as an antihistamine but subsequently shown to elicit antispermatogenic effects (i.e., germ cell loss from the seminiferous epithelium and testis atrophy) in mice, rats, dogs and monkeys [56–62]. In the rat, CDB-4022 (a racemic mixture of *l* and *d* isomers) induced irreversible infertility [61], but in the monkey *l*-CDB-4022 caused reversible oligospermia [63], illustrating variability in the way different species respond to CDB-4022. Interestingly, infertility was reversed in the rat when the administration of CDB-4022 was preceded by the administration of a GnRH agonist or antagonist to suppress endogenous testosterone [60,62]. CDB-4022 appears to target the Sertoli cell [59], as evidenced by the many parameters of Sertoli cell function that were shown to be affected within hours of CDB-4022 administration. For example, there was a significant decrease in serum inhibin B and FSH in rats treated with *l*-CDB-4022, suggestive of a disruption in spermatogenesis, but activin A, testosterone (however, in another study, serum testosterone was shown to decline in CDB-4022-treated adult rats [64]) and LH remained unchanged [60,61]. Equally important, the micro-tubule network, which gives the Sertoli cell its columnar shape, was disrupted and the steady-state levels of several adhesion proteins (e.g., cadherin, catenin, nectin, afadin and integrin β1) were shown to change in the rat testis following *l*-CDB-4022 treatment [65]. In addition to these biochemical changes, *l*-CDB-4022 also activated the mitogen activated protein kinase (MAPK) pathway in that an increase in the levels of phosphorylated extracellular signal-regulated kinases 1 and 2 [ERK1/2] was observed [65]. The MAPK pathway is comprised of several serine/threonine kinases that respond to extracellular stimuli and control diverse cellular events such as mitosis, differentiation, proliferation and cell survival [66–69] so that *l*-CDB-4022 likely targets some important aspect of Sertoli cell function that inadvertently leads to germ cell loss from the seminiferous epithelium.

## 2.5. Analogues of indazole-3-carboxylic acid

**2.5.1. Adjudin—**Adjudin [1-(2,4-dichlorobenzyl)-1*H*-indazole-3-carbohydrazide] is an analog of lonidamine [1-(2,4-dichlorobenzyl)-1*H*-indazole-3-carboxylic acid], a chemotherapeutic agent known to inhibit the aerobic glycolysis of tumor cells [70,71], that causes reversible infertility in rats, rabbits and dogs without affecting the hypothalamic-pituitary-testicular axis [10,11,13,14,72] (Fig. 1). In essence, adjudin affects fertility by disrupting Sertoli-germ cell junctions, and once this occurs, germ cells slough the seminiferous epithelium prematurely. Once adjudin is cleared from the systemic circulation (i.e., by 24–48 h) and its adverse effects in the seminiferous epithelium of the testis have culminated, fertility begins to bounce back gradually [i.e., by 82 days following two oral doses at 50 mg/kg body weight (b.w.) administered 1 week apart] [12]. This suggests that adjudin, at least at this dose, does not destroy spermatogonial stem cells. It is worth noting that cell adhesion was not affected in other organs when examined histologically [11,13]. Moreover, many of the significant gene and protein changes (e.g., *RhoB*) that were

demonstrated in the testis were not observed in other organs such as the brain and kidney [73]. When [$^3$H]-adjudin was administered to adult rats, less than 0.05% was uptaken by the testis [12]. Instead, the greatest percentage of [$^3$H]-adjudin that was recovered was from the serum (~3.5%), followed by the muscle (~0.8%), liver (~0.6%) and small intestine (~0.2%) 3 h after administration; and interestingly, less than 6% [$^3$H]-adjudin was recovered in total [12]. These results are interesting for another reason: oral administration of adjudin (50 mg/kg b.w. for 29 consecutive days) in a subchronic toxicity test performed by licensed toxicologists resulted in adverse effects in two of these organs, namely, muscle atrophy and liver inflammation, in male (but not female) rats [13]. Nevertheless, such adverse effects would preclude adjudin from becoming a marketable male contraceptive. In addition, adjudin was shown to have low bioavailability in the rat, but the cause(s) (i.e., first-pass metabolism, insufficient time for absorption in the gastrointestinal tract, hydrolysis by digestive enzymes, etc.) for this is not known. Micronization by pulverization, which decreased adjudin's particle size to ~50 μm, did improve dissolution rate in the gastrointestinal tract since only 16 mg/kg b.w. was needed to cause reversible infertility versus the original formulation which required 50 mg/kg b.w.

In order to circumvent adverse effects (i.e., muscle atrophy and liver inflammation) that were noted in the subchronic toxicity test, adjudin was delivered directly to the testis via conjugation to a recombinant FSH mutant (note: the mutant's hormonal activity, but not its ability to bind the FSH receptor present on the Sertoli cell surface, was deleted) which served as a "carrier." When this FSH mutant was used to deliver adjudin to the testis, reversible infertility was induced when 0.5 mcg adjudin–FSH mutant was administered intraperitoneally to adult rats, representing a substantial increase in selectivity and efficacy over the oral administration of adjudin at 50 mg/kg b.w. Moreover, this approach by-passed issues with toxicity and bioavailability since adjudin was delivered directly to the testis. However, this approach is prohibitively expensive for a male contraceptive because of the cost associated with the production of recombinant protein and its conjugation to adjudin. In line with this previous study, we are currently making good progress in appropriately modifying the original formulation of adjudin so that the effective dose needed to induce reversible infertility in rats can be lowered several-fold, thereby minimizing or even completely eliminating side effects noted in the muscle and liver in the subchronic toxicity test [13].

On a final note, a recently published study on drug transporters and adjudin has helped us to better understand how this compound enters and exits the testis [74]. While adjudin is a small, lipophilic compound (335.18 g/mol) that should easily cross the blood-testis barrier via diffusion, findings from a study using [$^3$H]-adjudin to assess the tissue distribution and bioavailability following its administration by gavage have shown that adjudin is not freely permeable to the blood-testis barrier [12]. Instead, it appears that adjudin is utilizing drug influx and efflux pumps present on the basolateral surface of Sertoli cells and at the site of the blood-testis barrier to enter and exit the seminiferous epithelium. In this recent study from our laboratory, the steady-state levels of several efflux pumps, namely permeability glycoprotein (P-glycoprotein, encoded by the *Abcb1* gene), multiple drug resistance protein 1 (encoded by *Mrp1* gene) and ATP-binding cassette sub-family G member 1 (encoded by

*Abcg1* gene) increased [74] at the time [$^3$H]-adjudin peaked in the testis (i.e., 3–24 h) [12]. Interestingly, we found that P-glycoprotein, which localized at the blood-testis barrier in untreated control testes, increased its association with other integral membrane proteins present at the blood-testis barrier, namely occludin, claudin-11 and junctional adhesion molecule-A (JAM-A) at the time [$^3$H]-adjudin was highest in the testis [74]. These results may suggest that an increase in P-glycoprotein association with occludin, claudin-11 and JAM-A occurs so that the integrity of the immunological barrier can be reinforced, thus prohibiting adjudin from entering the seminiferous epithelium. However, adjudin still enters the seminiferous epithelium to cause germ cell loss, possibly using a drug transporter that was not investigated in our study. If one can specifically "shut down" the drug pump (s) being used by adjudin to enter the muscle and liver, side effects may also be alleviated.

**2.5.2. Gamendazole**—Gamendazole [*trans*-3-(1-benzyl-6-(trifluoromethyl)-1*H*-indazol-3-yl)acrylic acid] (Fig. 1) is an analogue of indazole-3-carboxylic acid (selected from a screen of 150 lonidamine analogues) that was previously shown to affect spermatogenesis in different species [10,11,75,76] and to cause infertility in rats at single doses ranging from 1.5 to 25 mg/kg b.w. when administered orally or intraperitoneally [77]. For instance, infertility was achieved by 3 weeks in seven out of seven rats receiving a single oral dose of gamendazole at 6 mg/kg b.w., followed by the recovery of fertility which was observed by 9 weeks in four out of these seven animals. At 25 mg/kg b.w. of gamendazole, no gross histopathological changes (e.g., inflammation, necrosis, hemorrhage or tumors) were observed in any organ in all treated rats when compared to untreated rats. Similar to CDB-4022 and adjudin, it was concluded that the Sertoli cell was the primary target of gamendazole because this compound was shown to decrease the level of serum inhibin B in vivo, as well as to inhibit its production in Sertoli cells in vitro [77,78]. Importantly, gamendazole did not affect serum testosterone levels, but a transient increase in serum FSH was noted within 1 week of treatment [78]. Using proteomic and molecular techniques, Tash et al. [77] also showed that biotinylated gamendazole was able to bind to heat shock protein HSP 90-β (encoded by *Hsp90ab1* gene) and eukaryotic translation elongation factor 1 α 1 (encoded by *Eef1a1* gene) in the testis, as well as in Sertoli cells and ID8 ovarian cancer cells. Moreover, gene microarray experiments showed an up-regulation in interleukin 1 and nuclear factor κB inhibitor α in the testis within hours of gamendazole treatment [77], suggestive of inflammation and apoptosis since both genes are known to play active roles in these cellular processes [79,80]. H2-gamendazole (3-[1-(2,4-dichlorobenzyl)-6-trifluoromethyl-1*H*-indazol-3-yl]-propionic acid), another analogue of lonidamine, is also known to possess anti-spermatogenic effects but this compound remains to be tested for safety and efficacy.

In this respect, it is important to note that mortalities were noted in three out of five adult rats, representing 60% of animals dosed with gamendazole at 200 mg/kg b.w [78]. However, no deaths were reported when rats were treated with adjudin, even when a comparable single dose or consecutive doses were administered by gavage including a subchronic toxicity with 10 male and 10 female rats in which each adult rat received 50 mg/kg b.w. via gavage daily for 29 days (Ref. [12,13], Mruk and Cheng, unpublished findings). While the authors of the gamendazole study did not discuss in great detail why deaths were observed at this higher

dose, the trifluoromethyl group attached to the indazole ring of gamendazole may explain this compound's toxicity as this is one key difference between gamendazole and adjudin. Indeed, there are several examples in the literature in which the trifluoromethyl group is associated with severe toxicity (i.e., death). One example being hydramethylnon, a trifluoromethyl aminohydrazone and pesticide used in baits (i.e., Combat®) to control insects in both indoor and outdoor settings that disrupts cellular respiration by inhibiting the electron transport chain in mitochondria, thereby resulting in death usually within 3–4 days after treatment [81,82]. This compound was shown to be equally harmful to aquatic life; in rainbow trout, the $LC_{50}$ was estimated to 0.16 mg/L water 96 h after exposure. Interestingly, the testis was also shown to be a target for hydramethylnon. For example, mice fed daily for 18 months with this compound at 3.8 mg/kg b.w. developed severe testicular lesions, while in rats and dogs, testicular atrophy was also observed [83].

Gamendazole's toxicity may also be the result of the acrylic acid group as this is a second key difference between gamendazole and adjudin. Mortalities in two out of six adult rats, representing ~30% of animals, were also noted following administration of 200 mg/kg b.w. AF-2785, 1-(2,4-dichlorobenzyl)-1H-indazole-3-acrylic acid (Fig. 1), another lonidamine analogue that also contains an acrylic acid group at the indazole ring as well [78]. In a separate and unrelated subchronic study to assess the toxicity of acrylic acid, deaths were also reported in Wistar male and female rats: 50% of rats died when they were dosed orally with 150 mg/kg b.w. acrylic acid five times per week for 3 months, whereas this mortality rate increased to 75% when 375 mg/kg b.w. was administered via this same regimen [84]. At this point, it remains to be determined if rodents, but not other mammals such as rabbits and dogs, are especially sensitive to compounds containing one or both of these chemical modifications, thereby resulting in death in some animals, as species differences can exist. Interestingly, lonidamine which contains a carboxylic acid group did not produce a single mortality as previously observed by us and recently reported by Tash et al. [78]. Regardless, lonidamine is considered to be more toxic than adjudin; and while it is associated with several side effects, it is presently being used to kill tumor cells that have been sensitized by X-irradiation [85–87]. Thus, we conclude compounds having an acrylic acid group to be most toxic and even fatal if a high dose is used (i.e., gamendazole, AF-2785), immediately followed by compounds having a carboxylic acid group (i.e., lonidamine) and finally followed by those having a carbohydrazide group (i.e., adjudin). Nevertheless, much additional research is needed — research that not only focuses on gamendazole's mechanism of action but also on the screening of new indazole-3-carboxylic acid entities because a male contraceptive pill that can cause mortalities in approximately 60% of subjects, albeit when administered at a high dose, is not likely to sit well with the consumers, the general public and pharmaceutical companies.

## 3. Future perspectives and concluding remarks

In this review, we have discussed the current status of research relating to nonhormonal male contraception, including RISUG, contraceptive vaccines, $Ca^{++}$ channel blockers, indenopyridines and indazole-3-carboxylic acid analogues. While several of these approaches appear to show promise, additional research is needed so that a safe, effective, reversible and affordable male contraceptive can be brought to the consumer market. Of

these criteria, safety is the most important since male contraceptives would be used voluntarily by otherwise healthy individuals for extended periods of time. In recent years, there has also been increasing involvement to control wildlife (i.e., deer) and companion animal (i.e., cats and dogs) populations using approaches other than surgical sterilization which is costly, risky and difficult to perform in a field setting (as is the case for wildlife) so that male contraceptives that are safe but irreversible should not be disregarded as having no clinical use. At present, we remain hopeful that continued research will identify a nonhormonal contraceptive that is safe, effective, reversible and affordable in the near future.

## Acknowledgments

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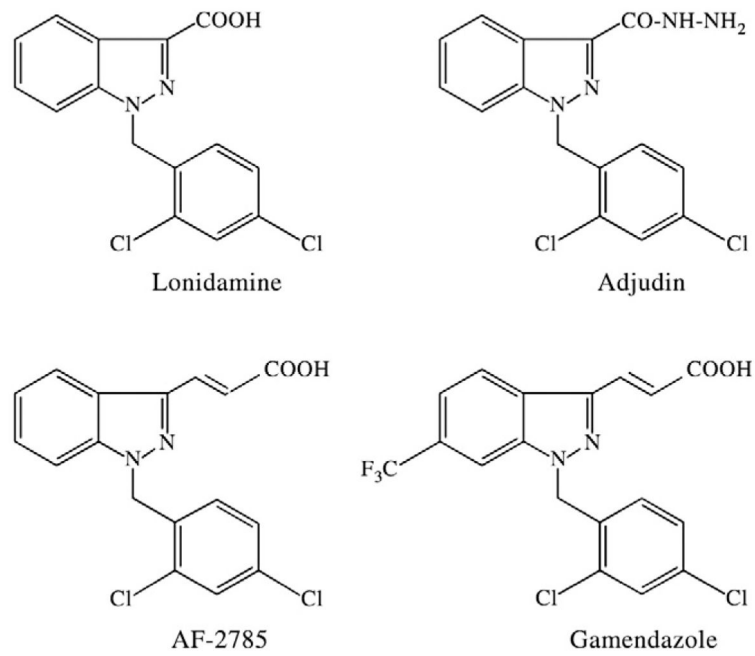
**Fig. 1.**
Chemical structures of Ionidamine, adjudin, AF-2785 and gamendazole.