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Review article

Non-steroidal interference with male fertility

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Introduction

The male gamete has long been thought to be the most vulnerable point at which to interfere with fertility, particularly when this could be accomplished non-invasively at the time of transfer from male to female reproductive tracts. Thus the earliest attempts to interfere with male fertility date back to Roman times [1]. They were mechanical in nature and depended upon prevention of intromission (infibulation) or use of a barrier (condom). Also from the beginnings of recorded history, materials have been inserted into the vagina which interact with spermatozoa to prevent fertilization. Early materials were employed empirically and consisted of acidic or sticky materials [2,3]. Early frank spermicidal materials included quinine and mercurials [4,5].

A milestone in the development of spermicides was the discovery in the early 1950s of the efficacy of the detergents typified by nonoxynol-9 [6]. More potent surfactants have been identified from time to time [7]. In the carefully monitored, well-motivated small-scale clinical trials these vaginal spermicides can achieve failure rates as low as 0.3 per 100 women years, essentially equivalent to the oral contraceptives for women [8–11]. Unfortunately, in widespread use, failure rates are much higher and retrospective surveys have shown up to 40% of users to become pregnant within 1 year [12–15]. With the exception of the animal studies reported on an imidazole with some additional loci of action [16–18] and some acrosin and hyaluronidase inhibitors [19–21], no noteworthy research has appeared in this area recently.

It is probable that attempting to nullify very large numbers of normal motile spermatozoa within the female tract and within the anatomical and time constraints involved is doomed to failure. A better approach to male contraception with lesser constraints would likely be through administration of agents directly to the male. The present review addresses the varied history of this approach as it applies to non-steroidal materials.

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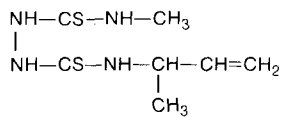
Inhibition of male fertility

Antispermatogenic agents

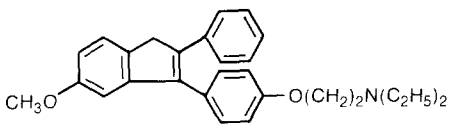
(i) Pituitary inhibition

Hormonally active agents The earliest recorded studies on control of male fertility by chemical means administered to the male capitalized upon the observation that administration of low doses of testosterone to male rats was antispermatogenic [22]. As we now know, this reflected sufficient circulating androgen to cause feedback inhibition of gonadotropin release but insufficient intratesticular androgen levels to maintain spermatogenesis directly [23–25]. Studies in men with testosterone or its esters, however, never consistently achieved azoospermia or protection from pregnancy, except at dose levels expected to change lipoprotein formation and/or blood cell formation [26–28]. The long history of attempts to use steroids as indirect regulators of spermatogenesis is outside the scope of this review and is described by Dr Nieschlag in his review paper [29].

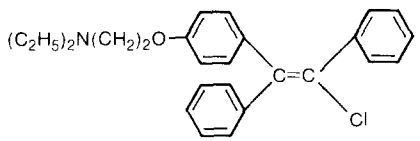
From time to time non-steroidal structures with hormonal activity have been described (Figure 1) and used to re-evaluate the potential for hormonal negative feedback [30–32]. Out of these agents, clomiphene and methallibure were tested in humans before being rejected due to side-effects which included nausea, dizziness and headache [33–35].



1-(α-methylallythiocarbamoly)-2
-(methylthiocarbomoly)-hydrazine
(methallibure; ICI 33, 828)



2[p-(6-methoxy-2-phenylinden-3-yl)
-phenoxy] triethylamine
(U-11, 555A)



2-[4-(2-chloro-1,2-diphenylethenyl)-phenoxy]-
N,N-diethylethanamine
(Clomiphene;MER-41)

Figure 1 Non-steroidal structures inhibiting pituitary gonadotropin release

Luteinizing hormone-releasing hormone analogs The possibility of achieving male contraception through pituitary inhibition has recently come back into vogue with the availability of luteinizing hormone-releasing hormone (LHRH) analogs. Agonist analogs, through a mechanism involving desensitization and down-

regulation of homologous pituitary receptors (and perhaps some direct testicular effects in rats) inhibit all testicular function, including spermatogenesis. Azoospermia was achieved in dogs with these agents [36] and although initial studies in monkeys using daily injection were unsuccessful [37], later trials using continuous administration of the analogs also reached azoospermia [38]. A similar success has not been achieved in men [39–41] and it now seems unlikely that the agonists will be clinically useful male contraceptives.

On the other hand, highly potent antagonistic analogs of LHRH have become available and, at least in animal studies, seem to be more reliable in inducing azoospermia [42]. If the concerns over enhanced toxicity and high dose requirements can be resolved, the LHRH antagonists may be the first successful male contraceptives acting via pituitary inhibition [43]. Of course any agent acting via this mechanism will require supplementation with androgens, and this in itself may pose a problem of interaction as testosterone can directly support spermatogenesis [44].

(ii) Direct testicular action

Starting in the early 1950s various series of non-hormonal agents were described which inhibited spermatogenesis by direct testicular action (Table 1).

Nitrofurans A group of bacteriostatic compounds, the nitrofurans (Figure 2) were shown to be directly antispermatogenic, some affecting primary spermatocyte division [45–47], while others acted at the level of the spermatogonia [48]. The compounds were required to be administered for several weeks at daily doses of 25–100 mg/kg orally, and the effects were reversible on cessation of treatment. There was reportedly no effect on either Sertoli cell or Leydig cell function [49], although a compensatory rise in pituitary gonadotropin was noted associated with the germ cell depletion [50]. The effects of one of these agents, furadoxyl, was prevented in rats by concomitant administration of cysteine [51], suggesting that the effect was mediated through sulfhydryl enzymes. However a similar concomitant treatment with cysteine does not reverse the antifertility in mice [52]. In trial the compounds proved too toxic for use in man, causing headache and gastrointestinal disturbances [53]. In addition, compounds of this series have monoamine oxidase inhibitory activity (furazolidone) and may induce peripheral neuropathies (nitrofurantoin) and mammary gland tumors (nitrofurazone).

Alkylating agents An extensive series of work was performed by Jackson [54–61] and others, mostly in rats but also in rabbits, dogs and monkeys [62–65] exploring the effects of alkylating agents on spermatogenesis. These agents varied in the locus of their effects from spermatogonia to late spermatids and some appeared to induce only functional male antifertility, perhaps by affecting epididymal maturation of spermatozoa. While it is known that these and other chemotherapeutic drugs are associated with infertility in man [66], use of such general cytotoxic agents for other than life-threatening disease is not warranted.

Thiophenes An isolated report showed 5-chloro-2-acetyl-thiophen (Figure 3) administered orally to rats at 0.5 mg/kg daily for 10 or more days produced reversible

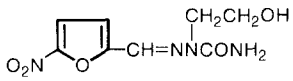
Table 1 Non-steroidal agents interfering with male fertility

Agent	*Year first reported**	*Action, site*	*References*	*Tested in man**	*References*
Nitrofurans	1950	antispermatogenic, testis	45	yes	53
Alkylating agents	1952	antispermatogenic, testis	62–65	no	[66,282,283]
Thiophenes	1956	antispermatogenic, testis	67	no	
Gossypol	1957	sperm function, sperm maturation/epididymis antispermatogenic, testis	174,176	yes	183
bis-Diamines	1960	antispermatogenic, testis	68–73	yes	76,77
Methallibure	1961	antispermatogenic, pituitary	31	yes	35
Clomiphene	1961	antispermatogenic, pituitary	30	yes	23,34
Dinitropyrroles	1963	antispermatogenic, testis	79–82	no	
Fluoroacetamide	1964	antispermatogenic, testis	83–86	no	
1-substituted, 5-nitroimidazoles	1968	antispermatogenic, testis	87,88		
α-chlorohydrin	1969	sperm function, epididymis antispermatogenesis	133,134	no	
Organosiloxanes	1972	sperm function, epididymis antispermatogenic, testis	90,91	no	
Prostaglandins	1973	antispermatogenic, testis Sertoli cell (?)	94–96	no	
Pipecolinomethyl-hydroxyindane	1974	antispermatogenic, testis Leydig cell (?)	101	no	
5-thio-D-glucose	1975	antispermatogenic, testis	102	no	
Indazole-3-carboxylic acids	1976	antispermatogenic, Sertoli cell	119–122	yes	127
Indenopyridines	1977	antispermatogenic, testis	129–131	no	
6-halo-6-deoxysugars	1978	sperm function, epididymis	153–156	no	
LHRH analogs	1978	antispermatogenic, pituitary, testis (?)	284	yes	39–43
Sulphasalazine	1979	sperm maturation, epididymis (?); testis (?)	170–172	no	[165–169]
Phenoxybenzamine	1984	block to ejaculation, vas deferens	222	yes	221,223
Papaya seed extract	1985	sperm maturation, epididymis	280,281	no	
1-substituted imidazoles	1985	sperm function, accessory organs; sperm	227	no	

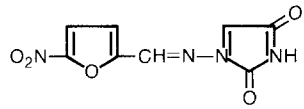
* As antifertility agents
[ ] indicates references in which antifertility effects in man are documented.

inhibition of spermatogenesis at the level of the primary spermatocytes [67]. Neither spermatogonia nor Sertoli cells were affected. These agents did not progress to trials in men due to unacceptable levels of toxicity [48].

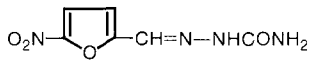
Bis-diamines One of the approaches which came closest to a marketed male contraceptive derived from a series of amoebicidal compounds (Figure 4), shown to have antispermatogenic activity. The male antifertility activity of these bis(dichloroacetyl)diamines did not parallel the amoebicidal activity. The compounds produced reversible inhibition of spermatogenesis in monkey, rat, mouse, dog and guinea pig by acting on spermatids and spermatocytes and with no effect on pituitary gonadotropins [68–73]. Studies in guinea pigs revealed that low doses



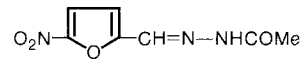
5-nitro-2-furaldehyde-2-(2-hydroxyethyl) -semicarbazone (Furadroxyl; Nidroxyzone)



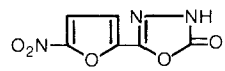
N(5-nitro-2-furfurylidene)1-amino -hydantoin (Nitrofurantoin, Furadantin)



5-nitro-2-furaldehyde semicarbazone (Furacin; Nitrofurazone)



5-nitro-2-furaldehyde acetylhydrazone (Nihydrazone)



5(5-nitro-2-furyl)-1,3,4-oxadiazoline-2-one (AF-4)

Figure 2 Structures of representative antispermatogenic nitrofurans

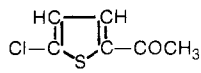
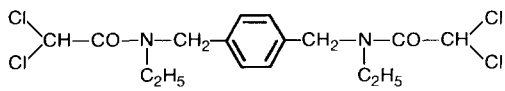
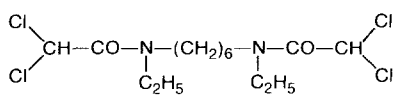


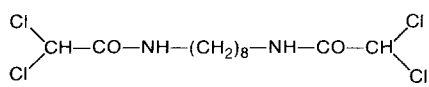
Figure 3 Structure of 5-chloro-2-acetyl-thiophen



N,N'-bis(dichloroacetyl)-N,N'-diethyl-1,4-xylenediamine (WIN-13, 099)



N,N'-bis(dichloroacetyl)-N,N'-diethyl-1,6-hexamethylenediamine (WIN-17, 416)



N,N'-bis(dichloracetyl)-1,8-octamethylenediamine (WIN-18, 446)

Figure 4 Structures of representative bis-diamines

over a 2-week period permitted normal development of spermatids up to the cap phase of acrosomal development [74]. Subsequently there was distortion of the acrosome and the nucleus. The misshapen sperm were then either retained and underwent degeneration or were released but incapable of fertilization. Their antispermatogenic effect is associated with inhibition of hyaluronidase, interference with utilization or formation of lactic acid and bicarbonate and the induction of steatogenesis and tissue hypoxia [75]. Both dose requirement and time to effect varied with species. The minimal effective daily dose in the rat was 50 mg/kg and effects were noted at 2 weeks; the dog required 150 mg/kg daily and the monkey 250 mg/kg daily, and 16 and 14 weeks of dosing were required, respectively. The effects were limited to the testis and the compounds were remarkably non-toxic [68].

The compounds were tested by twice-daily administration for a year to men (male inmate volunteers at the Oregon State Penitentiary) [76,77]. Azoospermia was reached in 10 weeks and was essentially maintained for the duration of treatment. Complete reversal occurred within 14 weeks of cessation of dosing. Unfortunately the compounds, through their inhibitory effect on aldehyde dehydrogenase [78], resulted in severe symptoms (flushing, hyperthermia, dyspnea) in a manner resembling the effects of Antabuse® (disulfiram), in subjects who drank alcohol and development was discontinued. Although presumably this side-effect would not have been a problem in a subpopulation of candidates for male contraception, perhaps these effects would signal other more subtle changes in other enzyme pathways.

Dinitropyrroles In 1963 studies with a series of 1-substituted dinitropyrroles were reported [79]. These compounds, of which the most active was ORF 1616 (Figure

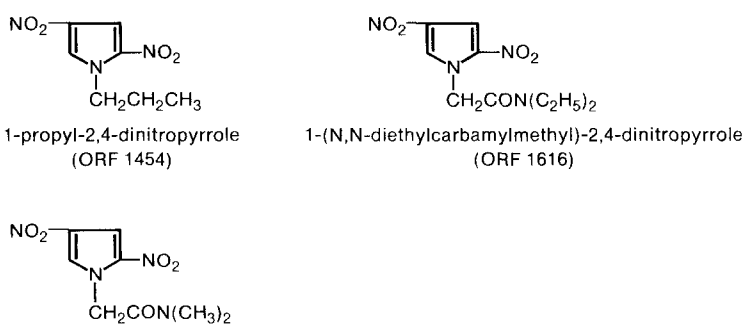
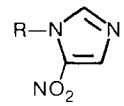


Figure 5 Structure of some antispermatogenic 1-substituted dinitropyrroles

5), inhibited spermatogenesis at the level of the spermatocytes and were effective in rats, guinea pigs and rabbits [80]. Daily doses of 40 mg/kg over 30 days reduced testis size in rats by 50%. A single oral dose of 500 mg/kg caused infertility in 3–7 weeks and complete recovery was noted by 11 weeks. Single doses of 500 mg/kg at monthly intervals maintained sterility over a 6-month period [81]. Daily doses up to 9 mg/kg were ineffective in rhesus monkeys [82]. These agents were neurotoxic in dogs [79] and did not proceed to human trial.

Fluoroacetamide In 1964 it was noted that oral administration of low doses (50 mg/kg of diet) of fluoroacetamide (CH_2FCONH_2), a rodenticide and insecticide with an oral LD_{50} in rats of 15 mg/kg, caused testicular atrophy in that species [83]. The effect was exerted directly at the testicular level and no effect on Leydig cell function was detected. Subsequent studies showed the effect to be completely reversible after discontinuing treatment [84]. More detailed later investigations revealed that daily oral doses of 5–50 μg per rat specifically inhibited the later stages of spermatid development [85,86]. Oral doses of 125–250 μg daily had a cumulative effect on the early stages of spermatogenesis and caused regression to spermatogonia and Sertoli cells only. No further studies have appeared.

1-substituted, 5-nitroimidazoles Patanelli reported, in 1963, on yet another series of heterocyclic compounds (Figure 6) with oral antispermatogenic activity in rats [87]. The most effective of these was 1-methyl-5-nitroimidazole with an MED of 200 mg/kg for a single oral dose. Fourteen days after such a dose in mature male rats spermatogenesis had ceased and many seminiferous tubules were populated only by Sertoli cells and spermatogonia [88]. The compound was thus acting at the

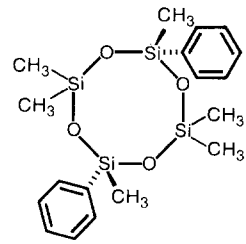


R = methyl; ethyl;
β-hydroxyethyl; β-acetoxyethyl;
β-hydroxypropyl.

Figure 6 Structural formulae of antispermatogenic 1-substituted, 5-nitroimidazoles

primary spermatocyte level. Pituitary and Leydig cell function were normal as judged by accessory organ weight and histology. In mating trials also conducted in rats a single oral dose of 250 mg/kg to the male caused substantially reduced fertility in the third to sixth weeks post-treatment, followed by recovery. On the basis of concomitant dosage and some protective action of cysteine, leucine and lysine, it was suggested that the mechanism of antifertility action was mediated through interference with protein biosynthesis in the testis. These compounds were not tested in man.

Organosiloxanes An organosiloxane (2,6-*cis*-diphenylhexamethylcyclo-tetrasiloxane; KABI 1774) (Figure 7) was noted to be antispermatogenic in rats and mice (10 mg/kg daily), rabbits (2 mg/kg daily) and dogs (10 and 250 mg/kg daily) by the oral route [89–91]. The compound was more effective by oral than the subcutaneous or intraperitoneal routes of administration. Activity was enhanced by the use of oleaginous vehicles. The effects were accompanied by atrophic changes in the Leydig cells and in the epididymides and accessory organs. It was suggested that the compound was inhibiting pituitary gonadotropin release by an estrogen-like action which would in itself abolish testicular steroidogenesis [91]. However, the possibility of an additional direct antiandrogenic effect at the epididymis was also suggested [90]. Large doses caused adrenal hyperplasia, hepatomegaly, decreases



2,6-cis-diphenylhexamethylcyclo-tetrasiloxane
(KABI 1774)

Figure 7 Structure of an antispermatogenic organosiloxane

in body weight and in serum levels of cholesterol, phospholipids and alkaline phosphatase [89,90]. These compounds were not evaluated in man.

Prostaglandins Seminal plasma is a rich biological source of prostaglandins (PG) and certainly the richest known mammalian source. There have been claims that an inverse relationship exists between seminal PG concentration and the percentage of abnormal spermatozoa in men attending a fertility clinic [92]. Intratesticular injection of either PGE_2 or $PGF_{2\alpha}$ in rats shuts down spermatogenesis and causes exfoliation of the germ cells by a mechanism suggested to be due to vascular effects [93]. Daily parenteral injection of 2 mg/kg PGE_1 or PGE_2 (Figure 8) caused an overall inhibition of spermatogenesis in rats and the presence of large numbers of exfoliated immature cells in the epididymides [94,95]. Similar effects have been noted in mice with both PGE and PGF analogs [96]. Intrascrotal administration of $PGF_{2\alpha}$ from silastic-polyvinyl pyrolidone tubes in rats and rabbits and continuous administration of 15(S)-15-methyl $PGF_{2\alpha}$ from silicone rubber discs disrupts spermatogenesis [97–99]. However, it is probable that the mechanism is mediated through interference with steroidogenesis [100], and this together with

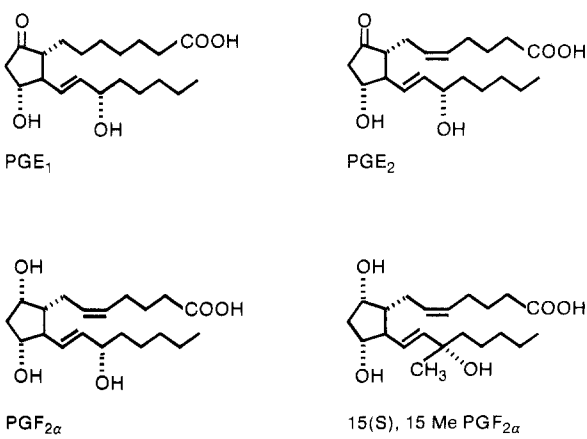
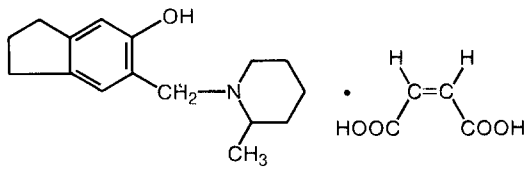


Figure 8 Structures of PGE_1, PGE_2, $PGF_{2\alpha}$ and 15(S), 15 Me$PGF_{2\alpha}$

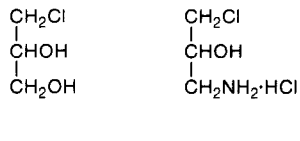
the adverse gastrointestinal side-effects of the PGs, makes it unlikely that the male antifertility effects will be capitalized upon.



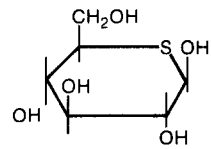
d,l-6-(N-α-pipecolinmethyl)-5-hydroxyindan maleate
(PMHI)

Figure 9 Structural formula of a testicular suppressive pipecolinomethylhydroxyindane

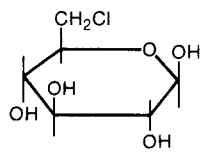
Pipecolinomethylhydroxyindanes Oral administration of a pipecolinomethylhydroxyindane (PMHI) (Figure 9) to rats at a daily dose of 1.5 mg/kg for 21 days caused testicular atrophy and reduction of prostatic weight [101]. Comparable results were obtained in rabbits, hamsters, guinea pigs, dogs and monkeys. The effects are mediated directly at the testis rather than via the pituitary. Addition of zinc chloride to the drinking water prevented the testicular effects in rats. Increasing the daily oral dose to 6.25 mg/kg caused irreversible sterility. This compound was not evaluated in man.



3-chloro-1,2-propanediol
(α-chlorohydrin; U-5897)



5-thio-D-glucose



6-chloro-6-deoxy-D-glucose

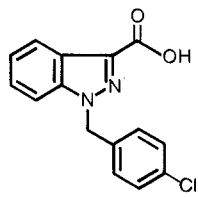
Figure 10 Structures of α-chlorohydrin, 1-amino 3-chloro-propanol hydrochloride, 5-thio-D-glucose and 6-chloro-6-deoxy-D-glucose

5-Thio-D-glucose Oral administration of 5-thio-D-glucose (Figure 10) to mice at the level of 30 mg/kg daily was reported to cause complete but reversible inhibition of spermatogenesis by interference with glucose transport and metabolism [102–104]. Early spermatids were most sensitive but in rats particularly loss of germinal epithelium was noted in some tubules. More detailed evaluation of the antispermatogenic effects in mice revealed only a partial reversal [105]. In rats permanent sterility resulted in all (25 mg/kg or more) or part (12.5 mg/kg) of the

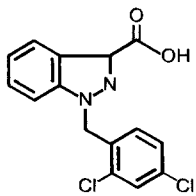
test group even though in the latter case 14 weeks of daily dosing were required to produce infertility [106]. Little or no effect is seen in hamsters [107]. Although it has been suggested that 5-thio-D-glucose has potential for human use [108], on the basis of permanent sterility and the potential for diabetogenicity, others are not so sanguine [109].

In this regard, it is of interest to note the high incidence of sexual dysfunction in male diabetics [110–112]. These include impotence and disturbed ejaculation often held to be mediated through a neuropathy [113]. However, there are many features suggesting a secondary hypothalamic hypogonadism including reduced circulating gonadotropin and testosterone levels, and hypospermatogenesis [112]. Seminal volumes, fructose levels and sperm motility are lowered [112]. Many of these results are duplicated in both spontaneous and induced animal models of diabetes mellitus [114–117], and result in infertility [118].

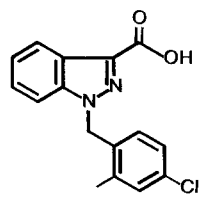
1-Substituted indazole-3-carboxylic acids The first member of this class of antispermatogenic agents to be found active was AF 1312/TS (Figure 11) [119–121]. However, more potent but relatively less toxic analogs were synthesized and studied extensively [122]. In rats effects are observed very quickly, even following a single oral dose of 50 mg/kg [120,123–125]. Histologically, alterations of Sertoli cells are first noted within 12 h and reach a maximum in 5–10 days causing germ cell exfoliation into the lumina of the seminiferous tubules. Neither LH levels nor Leydig cell function are affected; FSH levels may be elevated with length of treatment and/or degree of damage (probably due to interference in inhibin feedback).



1P-chlorobenzyl-1H-indazole-3-carboxylic acid
(AF 1312/TS)



(2,4-dichlorobenzyl)-1H-indazole-3-carboxylic acid
(Lonidamine; AF 1890)

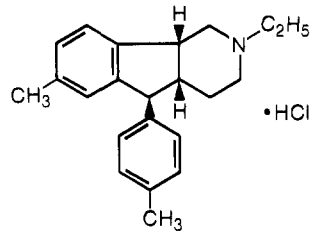


1-(4-chloro-2-methylbenzyl)-1H-indazole-3-carboxylic acid
(Tolnidamine)

Figure 11 Structures of representative 1-substituted indazole 3-carboxylic acids

Spermatogenesis begins to recover after 15–20 days without treatment [126]. In the dog effects on Sertoli cells are not seen. Instead, there is an arrest at late phases

of spermatid maturation [127]. Effects in man are said to resemble those in dog [cited in 120]. Impairment of renal function is reported for monkey but not rat or dog [128] and so far such effects are not reported in man [127].



±(4aRS,5SR,9bRS)-2-ethyl-1,3,4,4a,5,9b-hexahydro-
7-methyl-5-p-tolyl-2H-indeno(1,2-c)pyridine hydrochloride

Figure 12 Structure of an antispermatogenic indenopyridine derivative

Indenopyridines A low daily dose (30 mg/kg) of an indenopyridine (20–438; Figure 12) was used to regress seminiferous tubules to spermatogonia and Sertoli cells – only in 2 weeks in rats and dogs [129]. Infertility was complete in 3–4 weeks after onset of dosing. Full recovery was demonstrated after 12 weeks with no treatment in dogs but only after 25 weeks following a single low dose in mice. There were no signs of effects on pituitary function or on Leydig cells [130] and neither libido nor ejaculation were affected. A single high dose in mice (10 mg/kg) or rats (50 mg/kg) caused long-lasting/irreversible suppression of spermatogenesis by acting on the spermatogonia [131]. As with many other antispermatogenic agents, an exfoliation of germ cells into the seminiferous tubular lumen was noted very rapidly after treatment [131]. There was no sign of mutagenic effects with this compound. It is presumed that the therapeutic ratio was too slim to proceed into man.

Even if any of these testicular-active agents should be capable of inducing complete but reversible azoospermia in man, a major drawback to their use is the time elapsing to infertility and to reversal of effect once treatment is stopped, unless very late stages of spermatogenesis are specifically affected [132]. In addition, fears of mutagenic effects always exist for compounds acting during meiosis. A more appealing agent is one with a more rapid onset and reversal of activity. By definition such an agent must act at a post-testicular site.

Agents acting at post-testicular sites

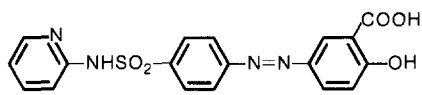
(i) Epididymal action

Alpha-chlorohydrin In 1969 the first compound to exert its male antifertility effect as soon as 7 days after beginning administration, and therefore acting at the level of the epididymis, was reported [133,134]. It was established that the interference with fertility in rats at low doses of α-chlorohydrin was due to suppression of

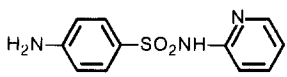
sperm motility, but not fertilizability [135]. The compound was not effective in mice and rabbits [136,137]. Administration of one or more high doses to rats led to formation of spermatoceles in the epididymis; the consequent back pressure on the testis caused cessation of spermatogenesis and permanent sterility resulted [138,139]. It was suggested that this compound might also be an alkylating agent [140], however there was no finding of alkylation of genetic material in dominant lethal studies [141,142]. Such an alkylating action may however have accounted for the finding of toxicity in monkeys due to bone marrow suppression [143]. A number of analogs were synthesized including 1-amino-3-chloro-2-propanol [144–146]. The latter compound was, unlike α-chlorohydrin, effective in mice, less toxic in laboratory animals and at first reported to be active in monkeys without bone marrow toxicity [147]. However, it was later determined to have unacceptable central toxicity in primates [148,149]. From studies of this compound and α-chlorohydrin it was established that the toxicity of the previously studied racemic mixture was more strongly associated with the *R*-isomer, and that the *S*-isomer was the compound of interest for male antifertility [150]. Although many animal studies were reported [151], a sufficient therapeutic index could not be confirmed and further development of these compounds for human use ceased. Alpha-chlorohydrin is presently marketed as a rodenticide, making use of the permanently sterilizing effects of the compound [152].

6-Halo sugars Some years later a series of 6-halo-6-deoxysugars were also reported to have antifertility effects, due to an action at the epididymal level, in rats, mice and marmosets [153–156]. Again, the effect was traced to an immobilization of the sperm following ejaculation due to an inability to generate ATP [157–159], through an inhibition of glyceraldehyde-3-phosphate dehydrogenase [160]. Unfortunately, the ability to block glucose metabolism was not specific to the epididymis and high doses led to central neurotoxicity and hemiplegia in mice [161], although doses as high as 240 mg/kg in rats had no toxicity other than induction of spermatoceles [162]. These compounds did not proceed to clinical testing in man.

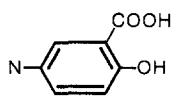
Sulfasalazine In 1979 the first reports appeared [163,164] on an infertility and associated oligospermia in men due to sulfasalazine (Figure 13), a drug which had



2-hydroxy-5[[4-[(2-pyridinylamino)sulfonyl]phenyl]azo] benzoic acid
(Sulphasalazine)



4-amino-N-2-pyridinylbenzenesulfonamide
(Sulphapyridine)



5-amino-salicylic acid

Figure 13 Structure of sulfasalazine and its component molecules

been on the market for a quarter of a century for treatment of ulcerative colitis. Further reports appeared and it is now accepted that male infertility is a frequent complication of sulfasalazine therapy [165–169]. Although there is an associated fall in sperm count to about 50% of normal, the major cause of infertility would seem to be due to depressed sperm motility and function and the increase in abnormal morphological forms, especially the characteristic 'megalo' head form. A dose-related antifertility effect has been found in rats [170]. However, even at 617 mg/kg inhibition of fertility was not complete and litter sizes of two or three were still achieved. The mechanism of action is still not known; no direct interference with sperm metabolism has been demonstrated. Sperm, recovered from hamsters fed sulfasalazine, poorly penetrated hamster eggs *in vitro* [171]. When female rats bred to treated males were evaluated 24 h after mating, percentage of recovered ova at the two-cell stage was significantly reduced, indicating interference with fertilization. In rats all effects noted for sulfasalazine can be duplicated with sulfapyridine [170–172]. It was suggested that some of the effects of sulfasalazine could be due to its ability to inhibit intestinal folate transport and folate reductase systems. However, large doses of folate could not reverse the effect of sulfasalazine [173]. Sperm function in sulfasalazine-treated men is impaired as judged by their ability to penetrate zona free hamster eggs *in vitro* [170]. It has been suggested that the enlarged (megalo) sperm head observed in treated men may be the result of changes in the cell membrane, and that this may correlate with the poor fertilizability of the sperm.

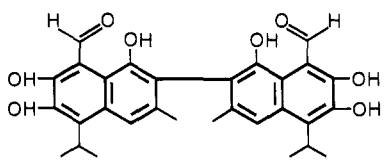


Figure 14 Structure of binaphthalene aldehyde tautomer of gossypol

Gossypol No review on non-steroidal interference with male fertility would be complete without reference to gossypol (Figure 14). This terpenoid from the cotton plant was established to have male antifertility activity, following elucidation of the cause of long-standing infertility in some districts in China where cooking with crude cotton-seed oil was prevalent [174–176]. The findings in man were succeeded by extensive animal studies; rats, hamsters and monkeys are sensitive to the antifertility action [177–184], mice and rabbits are poorly or not sensitive [185–187]. A successful clinical trial in man has been reported [188]. An analysis of the very extensive literature on gossypol is beyond the scope of this review; excellent reviews do already exist [174,176,184,189–191]. Suffice it to say that the definitive mechanism of inhibition of fertility has not been established, although a variety of effects of gossypol ranging from testicular to epididymal to direct action on spermatozoa have been reported (Table 2). A Leydig cell depression has been noted which correlates with a lowering of the serum testosterone : LH ratio [192]. Azoospermic individuals show an elevation of serum FSH levels; probably

Table 2 Mechanisms and sites of action of gossypol which may be involved in its antifertility action

Mechanism	*Interval to effect*	*Site of action*	*Species*	*References*
Antispermatogenic	4–5 weeks	spermatids, spermatocytes	rats, hamsters, rabbits, monkeys, men	202–204
Antispermatogenic	—	pituitary inhibition	rats	205
Mitochondrial damage	—	spermatids, spermatocytes	rat, man	206,207
Mitochondrial damage	30–50 days	spermatozoa	man	208
LDH-X inactivation	30–50 days	spermatozoa	mouse, man	209,210
Inhibition of sperm motility	*in vitro*	spermatozoa	man	211
Inhibition of sperm motility	2 weeks	spermatozoa	man	204
Inhibition of capacitation	2–5 weeks	spermatozoa/epididymis	guinea pig	212
Inhibition of acrosin	*in vitro*	spermatozoa	boar	213
Inhibition of ATP	*in vitro*	spermatozoa	boar	214
Inhibition of testicular steroidogenesis	*in vitro*	Leydig cell	rat	216
Inhibition of testicular steroidogenesis; elevation of LH levels	1–3 months	Leydig cell	rat, rabbit, man	192, 216–219
Inhibition of ABP production	*in vitro*	Sertoli cell	rat	220

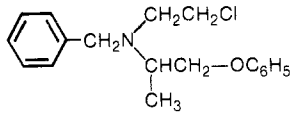
an effect rather than a cause [193]. In addition, the intriguing observations that gossypol can inhibit the lipoxygenase pathway [194] and that aspirin can antagonize the antifertility action [195,196] suggest a prostaglandin involvement. It may be that the antifertility effect is the resultant of several actions.

It is commonly believed that the toxic effects of gossypol [197,198], including hypokalemia, and the irreversible inhibition of spermatogenesis which develops with time and dose [199], precludes the development of this agent for use in the Western world, if not in China [192]. There are synthetic programs ongoing [200] which aim to separate the desired contraceptive effect from generalized toxicity, probably by concentrating on the (−)-epimers [201].

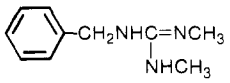
(ii) Post-epididymal action

Phenoxybenzamine It has been recognized for some time that many classes of therapeutic drugs, particularly CNS and autonomic system active agents, can affect sexual function [214]. The antihypertensive, phenoxybenzamine (Figure 15), was evaluated for treatment of premature ejaculation in men [221]. The success in its use for this indication was followed by claims of effective contraception in men due either to a lack of, or a retrograde, ejaculation [222,223]. Orgasm was reportedly still experienced even in the absence of ejaculation. It is unlikely that this, or other cardiovascular active agents [224–226], would ever be seriously considered for this contraceptive use.

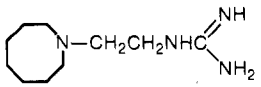
1-Substituted imidazoles Recently a series of experiments has been published leading to the possibility of orally administered, same-day active, male contraception [227]. They were based upon the observations that a variety of substances, in



N-(2-Chloroethyl)-N-(1-methyl-2-phenoxyethyl)
benzenemethanamine
(Phenoxybenzamine)



N,N'-Dimethyl-N''-(phenylmethyl)-guanidine
(Bethamidine)



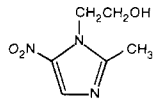
[2-(Hexahydro-1(2H)-azocinyl)ethyl] guanidine
(Guanethidine)

Figure 15 Structures of antihypertensive agents reported to interfere with ejaculation but not orgasm

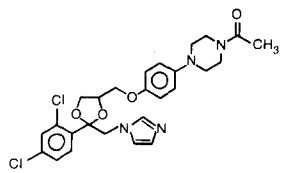
particular the nitroimidazoles (Figure 16), could rapidly appear in seminal plasma after oral administration [228–234]. Other studies demonstrated the spermicidal activity of a structurally related series of 1-substituted imidazoles [16–18,235,236]. This suggested that orally administered spermicidal compounds could appear in seminal plasma. Follow-up of the original examples was not successful as the nitroimidazoles were not spermicidal, and the highly effective spermicides were too rapidly metabolized *in vivo*. However, ketoconazole was identified as a compound with good pharmacokinetics and low spermicidal potency [237]. Oral administration of 1 g of this compound to dogs, rabbits or monkeys caused rapid immobilization of the sperm in ejaculates obtained 4 or more hours after dosing. The effect was correlated with presence of unmetabolized ketoconazole in the seminal plasma, although the levels were lower than those required *in vitro* to immobilize sperm. The observed CNS depressant effects, the induced emesis and suppression of testicular steroidogenesis together with other clinically noted toxicity [238–242] militated against further study of ketoconazole. Screening studies, however, identified two series of compounds; ketoconazole analogs and also some carbamates, which were orally active at doses of 10–30 mg/kg in dogs and which immobilized sperm in ejaculates obtained 4–48 h from treatment [243]. Breeding of the treated dogs did result in pregnancy, subsequently ascribed to the secretory profile of the compounds (as determined from split ejaculates) and to timing from insemination to fertilization in dogs [227]. Breeding trials in species which more closely resemble man have not yet been reported.

Concluding remarks

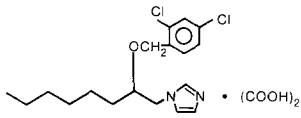
The present review amply demonstrates, contrary to popular belief, that the achievement of a male contraceptive has been a major scientific goal for the past



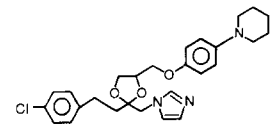
2-methyl-5-nitroimidazole-1-ethanol
(Metronidazole)



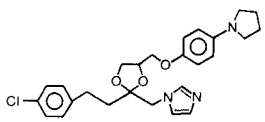
(±)-cis-1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)1,3-dioxolan-4-yl]methoxy]phenyl]piperazine
(Ketoconazole)



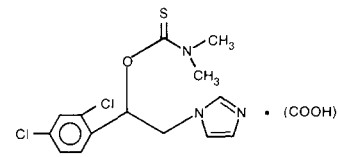
-1-[2-(2,4-dichlorobenzyloxy)octyl]imidazole hydrogen oxalate
(RS-37367)



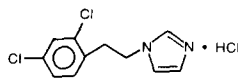
(±)-cis-2-[2-(4-chlorophenyl)ethyl]-2-[1H-imidazol-1-ylmethyl)-4-[(4-piperidinophenoxy)methyl]-1,3-dioxolane
(RS-29984)



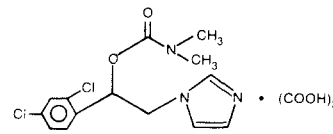
±)-cis-2-[(4-chlorophenyl)ethyl]-2-[1H-imidazol-1-ylmethyl)-4-[(4-(N-pyrrolidinophenoxy)methyl]-1,3-dioxolane oxalate
(RS-29636)



(±)-1-[2-(2,4-dichlorophenyl)-2-(dimethylaminothiocarbonyloxy ethyl]imidazole hydrogen oxalate
(RS-90847)



1-[2-(2,4-dichlorophenyl)ethyl]imidazole hydrochloride
(RS-41353)



(±)-1-[2-(2,4-dichlorophenyl)-2-(dimethylaminocarbonyloxy) ethyl]imidazole hydrogen oxalate
(RS-68287)

Figure 16 Structure of some 1-substituted imidazoles assessed for spermicidal activity. (From [227])

35 years. Seemingly, most of the theoretically possible sites of action have been explored [244]. They range from inhibition of spermatogenesis via the pituitary gonadotropins to direct testicular effects on specific cell divisions. Spermatozoal maturation and epididymal function have been attacked; as have sperm motility, metabolism and fertilizability. Even the ejaculatory process itself has received attention. Lest we should think that we have exhausted all possibilities, however, it should be noted that work on specific inhibition of FSH with the testicular-derived polypeptide inhibin continues [245] and novel, non-steroidal inhibitors of androgen binding protein are being reported [246]. In addition, the early studies on the antispermatogenic effects of minerals [247–268] have been succeeded by

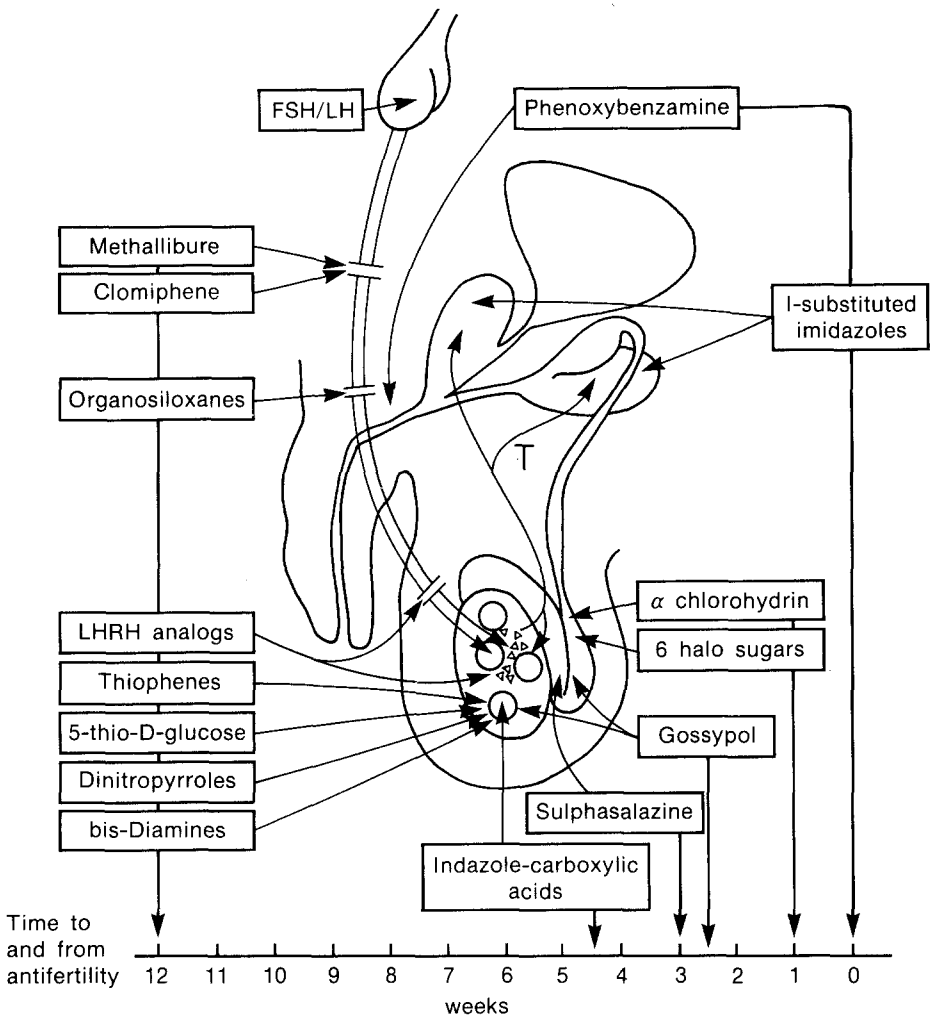


Figure 17 Schematic representation of site(s) of action and time, to or from dosing, to expression of, or recovery from, fertility inhibition with some representative non-steroidal male contraceptive agents

single bilateral intratesticular injection of glycerol [269]. Although Leydig cell function is not affected, the apparent irreversibility of the antispermatogenic effect, and concerns about the reliability of induction of complete antispermatogenesis, not to mention aesthetic considerations, probably rules out this type of approach.

It is of interest that with time attention has shifted from the testis to later and later post-testicular phases, culminating in the recently described materials which accumulate in accessory organ secretions to be mixed with sperm only at the moment of ejaculation [227]. Such a shift has not only dramatically shortened the latency to onset of and recovery from antifertility (Figure 17) but would minimize earlier worries of mutagenicity potential.

Leads for male contraceptive agents have arisen from varied sources including targeted screening programs in animals, belatedly recognized side-effects of commonly used therapeutic agents in man [163,164] or even the linking of infertility with a crude preparation of a cooking oil [174]. Similar opportunities to capitalize on side-effects of therapeutic agents may still await us [270–273]; some of the undesired side-effects noted for certain pesticides and nematocides are obvious starting points for male contraceptive development [274,275] and plant sources have not yet been exhausted [276–279].

At the present time there are more potential male contraceptive agents in clinical trial or under animal evaluation than at any previous time in history. Such intensified activity can only increase the possibility of achieving the much needed and long awaited goal of a contraceptive for men.

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