Ann Rev. Pharmacol. Toxicol. 1978. 18:167–87

ENDOGENOUS ANTISPERMATOGENIC AGENTS: PROSPECTS FOR MALE CONTRACEPTION

❖6711

L. L. Ewing
Department of Population Dynamics, Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland 21205

B. Robaire
Department of Pharmacology and Therapeutics and Department of Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada

INTRODUCTION

Interest in antispermatogenic agents has burgeoned in recent years because of the increased interest in the development of a systemic male contraceptive. Numerous excellent reviews have been written about such agents during the past decade (1–6). Perusal of these reviews leads one to the conclusion that a variety of antispermatogenic agents exist. Unfortunately, the reader soon realizes that most antispermatogenic agents cannot be exploited in the development of a male contraceptive because some are nonspecific cytotoxic agents, others are mutagenic and/or carcinogenic, and yet others exhibit deleterious side effects such as the Antabuse® effect of the bis-(dichloroacetyl)-diamines.

In this review, we emphasize some natural control points in spermatogenesis, elucidate which control points might be exploited in the development of antispermatogenic agents, and note the possible subtle interference with the intricate spermatogenic process resulting from the use of naturally occurring compounds.

Comprehensive reviews by Clermont (7) and Roosen-Runge (8) showed that spermatogenesis is a highly ordered, sequential series of cellular divisions and stages of differentiation that occur at a species-specific and fixed rate. We now appreciate that this spermatogenic process occurs in a unique environment (9) created in part by the intimate relationship resulting from occluding junctions between adjacent Sertoli cell membranes; these junctions separate the seminiferous tubule into basal

0362-1642/78/0415-0167$01.00

and adluminal compartments. Highlights of spermatogenesis include (*a*) the initial mitotic divisions of undifferentiated stem cells in the basal compartment; (*b*) the setting aside in this basal compartment of stem cells that may participate in the reinitiation of the spermatogenic process; (*c*) the movement of the rapidly proliferating cells destined to become spermatozoa into the adluminal compartment of the seminiferous tubule; (*d*) the completion of meiosis in the adluminal compartment; and (*e*) the metamorphosis of the prospective gamete (round spermatid) into the spermatozoon. Many of the above events occur only if the germinal cells are under the influence of steroid and protein hormones derived from either the nearby Sertoli and Leydig cells or the distant, but equally important, adenohypophysis. Thus, spermatogenesis is an intricate process that depends on a precise physical and chemical environment.

CONTROL OF SPERMATOGENESIS

Intratesticular Factors

EVIDENCE FOR A SPERMATOGONIAL CHALONE: EFFECT OF AQUEOUS EXTRACTS OF TESTICULAR TISSUE ON SPERMATOGONIAL DIVISIONS Epidermis, lung, liver, and numerous other tissues contain substance(s) that regulate(s) mitosis within each tissue. Such mitotic regulators were termed *chalones* by Bullough (10). By definition chalones are tissue-specific, species-nonspecific, noncytotoxic, and reversible in nature (11). Chalones are thought to be glycoproteins or peptides depending on their tissue of origin.

The testis is a likely source of chalone(s) since it contains an active germinal epithelium with numerous cell types in a variety of stages of differentiation. Primitive undifferentiated cells (stem spermatogonia) divide to form the differentiated spermatogonia which in turn give rise to spermatocytes which undergo meiosis to form spermatids. All differentiated spermatogonia are sensitive to X irradiation but stem spermatogonia exhibit a heterogeneous sensitivity to irradiation; that is, some stem cell types are destroyed by moderate doses of X irradiation (100-600 r) whereas others survive even 1500 r. Dym & Clermont (12) made the important observation that the radioresistant stem (reserve) spermatogonia began to divide rapidly after depletion of the radiosensitive cells in the germinal epithelium by X irradiation. Interestingly, this stem cell division stopped after repopulation of the seminiferous tubule. Such indirect evidence prompted these investigators to suggest the presence of a spermatogonial chalone.

The first direct evidence for a testicular spermatogonial chalone was provided by Clermont & Mauger (13) in 1974. These investigators demonstrated that a saline extract of normal adult testis contained a substance that inhibited specifically the proliferation of radioresistant stem spermatogonia in the irradiated rat testis. This water-soluble testicular extract caused a decrease in the incorporation of radioactive thymidine into stem but not differentiated spermatogonia when injected into irradiated adult rats. In addition, this substance was found to be noncytotoxic and tissue-specific. These findings have since been confirmed by Clermont & Mauger (14) in immature rather than X irradiated adult rats. McLaren (15) recently ex-

tended the above findings to show that the testicular extract was exercising its inhibitory effect on the division of both the radioresistant and radiosensitive stem spermatogonia but not the radiosensitive differentiated spermatogonia.

EFFECT OF SERTOLI CELLS ON SPERMATOGENESIS The Sertoli cell rests on the basement membrane of the seminiferous tubule and extends filamentous cytoplasmic ramifications towards the lumen of the tubule. Germinal cells are arranged between these Sertoli cell cytoplasmic projections. Spermatogonia and early spermatocytes are in the basal compartment near the basement membrane of the seminiferous tubule, while the more advanced spermatocytes and spermatids are arranged at successively higher levels in the adluminal compartment (9).

The interdigitation between Sertoli and germ cells has rendered the purification and characterization of each cell type from adult animals most difficult, and thus documentation of the precise functional role of Sertoli cells is lacking. Although our knowledge is incomplete, it is apparent that multiple spermatogenic control sites must exist between Sertoli and germ cells. Some of the probable components of this relationship are (*a*) the presence of specific, high affinity, membrane-bound receptors for follicle-stimulating hormone (FSH) on Sertoli cells, (*b*) the production or concentration by Sertoli cells of a soluble, high-affinity, androgen-binding protein (ABP) found in the seminiferous tubular fluid, (*c*) the production of a macromolecule by the seminiferous tubule that preferentially inhibits FSH secretion, and (*d*) the (probable) metabolism of certain steroids by Sertoli cells to 5α-reduced androgens and estrogens. Sertoli cell FSH receptors and the production of a macromolecule that inhibits pituitary FSH secretion are discussed later in this chapter.

Hansson and co-workers in 1972 (16) discovered a substance in the rat epididymis that bound androgens, particularly 5α-dihydrotestosterone (DHT) and testosterone with high affinity (10^{-8}–$10^{-10} k_d$). It has since been shown that this originated in the testis (17) and that its testicular content was reduced by hypophysectomy (18), restored by FSH (19), and maintained by either luteinizing hormone (LH) or testosterone alone (20). ABP has different physical and chemical properties from the intracellular androgen-dependent target organs such as the prostate (22, 23). In fact, the physicochemical and immunological characteristics of ABP from the rabbit epididymis were indistinguishable from those of rabbit testosterone-estradiol binding globulin (TeBG) [steroid binding globulin (SBG)], a protein found in the plasma of the peripheral circulation (24). Since the de novo biosynthesis of ABP from radioisotopic amino acids by testes has not yet been attempted, the question of whether ABP is synthesized by the testes or alternatively concentrated from circulating TeBG remains open.

The ascribed functional role for ABP is that of concentrating androgens in the adluminal region of the tubule, thus facilitating spermatogenesis (25). Though this hypothesis is very appealing because high concentrations of androgens are known to be present in seminiferous tubular fluid (26, 27) and needed for spermatogenesis (28, 29), an absolute requirement for ABP in any animal including man for the maintenance of spermatogenesis remains to be established. The steroidogenic capac-

ity of Sertoli cells in adult animals is a subject that is fraught with controversy (30, 31). It appears that these cells may convert certain steroids but that, in comparison to Leydig cell steroidogenesis, the capacity of Sertoli cells for this function may be quantitatively insignificant. However, some investigators have suggested that Sertoli cells of immature rats can convert testosterone to 5α-reduced androgens such as DHT and 5α-androstan-3α,17β-diol (3α-diol) (32). It is noteworthy that in hypophysectomized rats, 5α-reduced androgens can maintain spermatogenesis (33). Recently, Dorrington & Armstrong (34) showed that putative Sertoli cells obtained from immature rats and cultured in vitro produced radioimmunoassayable estradiol. Apparently these Sertoli cells cultured in vitro require testosterone or androstenedione as substrate and will not convert pregnenolone or other progestins to estradiol. These cultured cells respond to FSH, but not LH, with increased conversion of testosterone to estradiol. It has been suggested that Sertoli cell estrogen might influence Leydig cell production of testosterone since van der Molen and colleagues (35) have demonstrated estradiol binding sites in rat interstitial tissue. Such a control of Leydig cell function by Sertoli cell estradiol remains unproven at this juncture.

EFFECTS OF LEYDIG CELLS ON SPERMATOGENESIS The Leydig cell located in the interstitium immediately adjacent to the seminiferous tubule is thought to be the principal site of testosterone synthesis (36). The close spatial association of Leydig cells and seminiferous tubules may be important to provide a high local concentration of testosterone surrounding the germinal cells, because spermatogenesis fails when intratesticular testosterone concentrations are allowed to fall to levels normally found in peripheral blood. Peripheral blood levels that are sufficient to maintain all the androgen-dependent functions, e.g. accessory sex organ weights, are not sufficient to maintain spermatogenesis (28).

Testosterone may exert its biological activity only after it is converted to either 5α-reduced androgens or to estrogens in the target cells (37). There is strong evidence that androgen-dependent target organs such as the prostate activate testosterone by metabolizing it to dihydrotestosterone and 3α-diol (22, 23) while others such as the hypothalamus and adenohypophysis activate it by metabolizing it to estrogens (38). Evidence supporting the existence of similar mechanisms in the testis is that testosterone is converted to both 5α-reduced androgens and estrogens by testicular tissue (39, 40), that testicular tissue contains high-affinity, low-capacity intracellular binding proteins for both androgens and estrogens (19, 35), and that testosterone and 5α-reduced androgens will maintain spermatogenesis in hypophysectomized rats (33).

Central Nervous System Factors

HYPOTHALAMO-ADENOHYPOPHYSEAL CONTROL OF SPERMATOGENESIS

Luteinizing hormone Luteinizing hormone is a glycoprotein produced by the adenohypophysis which is distributed throughout the body via the vascular system. LH attaches to high-affinity membrane-bound receptors on the Leydig cell (41, 42).

This attachment is associated with a stimulation of adenyl cyclase activity which is thought to cause an increased conversion of cholesterol to pregnenolone leading finally to increased testosterone synthesis (43). Recently it has been shown that exposure of LH receptors to elevated LH concentration results in a loss of responsiveness to LH (44–46). This may be analogous to the desensitization of postsynaptic receptors which occurs after prolonged stimulation of nerve. It has been well demonstrated that testosterone can inhibit both the hypothalamic release of luteinizing hormone releasing hormone (LRH) (47) and the responsiveness of the adenohypophysis to LRH (48). Whether this negative feedback mechanism is normally affected in vivo at both sites or just at one is unknown (49). Furthermore, whether testosterone normally exerts these effects directly or only after conversion to estrogens or 5α-reduced androgens is controversial (50, 51).

Follicle-stimulating hormone The role of follicle-stimulating hormone in the adult male is unclear. Controversy has developed over the function of FSH for three reasons: (*a*) testicular products essential for spermatogenesis that are specifically elicited by FSH have not yet been elucidated, (*b*) the cellular site of action is difficult to prove, and (*c*) FSH effects on the testis are readily demonstrable only in immature testes. The present status of our knowledge suggests that FSH attaches to high-affinity receptors found on Sertoli cell membranes (52). This interaction then results in increased activity of both adenyl cyclase and protein kinase which leads to increased protein synthesis (52). We have already discussed the effect of FSH on ABP and estradiol concentrations in intact testes and Sertoli cell cultures. The fact that most of these effects of FSH are lost as rats gain sexual maturity suggests that FSH may be important only in the initiation of spermatogenesis (53).

Though gonadal steroids suppress both FSH and LH (54), LH is more readily suppressed than FSH by steroids. However, any steroid that suppresses one gonadotropin always suppresses the other (55). Interestingly, FSH and LH titers in peripheral blood of males of several species are dissociated as the result of specific experimental treatments or pathological conditions that directly inhibit spermatogenesis without inhibiting Leydig cell function (56). For example, idiopathic infertility in the human male is frequently characterized by azoospermia, normal LH and testosterone levels, but elevated FSH levels in the peripheral blood (56). Similar conditions can be produced experimentally in the rat by inhibiting spermatogenesis with antispermatogenic agents (57). These observations suggested the presence of some principle associated with the germinal epithelium of the testis that specifically regulated FSH secretion. McCullagh (58) postulated the existence of a water-soluble testicular substance that overcame the pituitary hypertrophy induced by castration without affecting accessory sex organ weights. This hypothetical material was named *inhibin* and has been prepared from ram rete testis fluid (59), bovine and human seminal plasma (60), bovine testis (61), and bovine follicular fluid (62). A number of investigators have attempted to purify and characterize this substance (56, 63–67); however, Franchimont and his collaborators (56, 66, 67) have apparently proceeded furthest towards these goals. They have successfully extracted a water-soluble material from bull and human seminal plasma that inhibits FSH

secretion in rats. When partially purified, this inhibitory principle retained its biological activity; did not bind steroids such as testosterone (T), DHT, and estradiol-17β; did not cross-react with antibodies to gonadotropins; and was susceptible to proteolytic enzymes. Thus, this substance is apparently proteinaceous and distinct from other macromolecules, such as LH and FSH, which are involved in the hypothalamo-adenohypophysealgonadal axis.

Considerable controversy has arisen regarding the cellular source of inhibin. Some investigators (68) believed spermatids to be the source, others (69) implicated Sertoli cells or spermatogonia, while a few (70) were unable to demonstrate any relationship between spermatogenic stages and FSH levels in peripheral blood. Steinberger & Steinberger (71) have substantiated the presence of a material that specifically regulates FSH secretion. In this latter study, the secretion by cultured pituitary cells of FSH but not of LH was markedly depressed by the addition of Sertoli cells or of spent media from these cells.

PINEAL CONTROL OF SPERMATOGENESIS The effect of the pineal gland on the male reproductive tract of species sensitive to photoperiodic stimulation has been clearly demonstrated, but no such marked effects have been found in males whose reproductive process is insensitive to photoperiod (72–74). Exposure of photoperiodic animals such as hamster to short-length days or blinding caused testicular involution (72, 75). However, this involution failed to develop if the male was subjected to pinealectomy (76). The testicular regression caused by light deprivation in the male hamster was accompanied by a progressive decline in plasma FSH, LH, and testosterone (77, 78).

Pineal indoles The pineal gland contains several indoles including 5-hydroxytryptophol, 5-methoxytryptophol, serotonin, and melatonin (79). Melatonin, which is found in highest concentrations in the pineal gland, has been reported not to have a gonadal effect (80), to have an antigonadal effect (81–83), and to prevent antigonadal effects in the male (84–86). These variable results are probably dependent on the species tested, the lighting regime to which the animals were exposed, the dosage of melatonin used, or the mode of melatonin administration. Turek et al (87) clarified the melatonin controversy when they showed that doses of melatonin, which caused antigonadal effects in hamsters exposed to long photoperiods, completely overcame the gonadal regression usually associated with diminished photic stimulation in male hamsters.

The mechanism by which melatonin inhibits spermatogenesis is unknown. Ellis (88) suggested that pineal indoles directly inhibit testosterone formation from radioisotopic pregnenolone. However, the physiological consequences of these findings must be questioned because many indoles, other than melatonin, will inhibit testosterone synthesis in vitro and because large concentrations of indoles were required to attain significant reductions in steroidogenesis (74). Melatonin has also been suggested to suppress the release of LRH (74, 89, 90) and/or to directly inhibit pituitary response to LRH (91). Finally, some investigators suggest that melatonin itself is not the antigonadal substance but that it merely mediates the release of a macromolecular pineal antigonadotropin (73, 85).

Pineal peptides Direct evidence suggesting that molecules of pineal origin, other than melatonin, affected gonadal function was provided by Benson and co-workers (92, 93). Orts and co-investigators (94–97) observed that extracts of bovine pineal caused regression of the reproductive tract in male and female mice. Subsequent experiments showed this pineal substance to be a peptide consisting of approximately six amino acids (98–101). Injection of similar pineal peptide preparations caused reduced serum LH and testosterone concentrations in addition to testicular regression in male rats (102).

Taken together, the above results clearly demonstrate that spermatogenesis is an intricate process that should be susceptible to interference at numerous sites by endogenous antispermatogenic agents.

ENDOGENOUS ANTISPERMATOGENIC AGENTS

Macromolecules

CHALONES It should be possible to arrest spermatogenesis by the systemic administration of a purified spermatogonial chalone. Such an antispermatogenic agent might afford several advantages: (*a*) mitosis in cells other than undifferentiated spermatogonia should be unaffected by the treatment since chalones, by definition, are highly specific (11); (*b*) the reserve undifferentiated spermatogonia should remain viable under the influence of the spermatogonial chalone for lengthy periods since these cells divide only rarely under normal circumstances (7); (*c*) the aspermatogenic state should be readily reversible upon removal of the chalone since undifferentiated spermatogonia have the capacity to completely repopulate the seminiferous tubule (7); and (*d*) such treatment should leave Leydig cell function unimpaired, thus insuring maintenance of potency, libido, and accessory sex organ function.

Numerous experimental problems must be overcome before the antispermatogenic characteristics of a spermatogonial chalone can be tested. The present assay for the spermatogonial chalone (13) is cumbersome and unsuitable for testing purification procedures. Therefore, the first order of experimentation might be to develop a simple, inexpensive, rapid, and accurate assay for spermatogonial chalone. One possible approach would be to isolate and culture undifferentiated spermatogonia with ^{3}H-thymidine, in the presence and absence of chalone. Obviously, considerable research would be required to isolate, to purify, and to obtain the chemical and physical characteristics of the chalone once the assay was developed. It is equally obvious that this interesting and important area of research should be pursued.

ANDROGEN BINDING PROTEIN The discovery, isolation, and partial purification of androgen binding protein (ABP) (16) leads naturally to the speculation that azoospermia might be produced by immunization of the male against ABP. However, this idea has certain limitations that detract from its potential use as a male contraceptive. First, it seems premature to suggest an antispermatogenic technique based on the neutralization of a molecule whose biological function is poorly understood (13). Second, since ABP is physically, chemically, and immunologically identical with testosterone-estradiol binding globulin (TeBG) (24) in the rabbit, a high

degree of cross-reactivity of the ABP antibodies with TeBG would be expected. Should this prove to be the situation in man, the ABP antibody might very well neutralize TeBG. Our third objection is that there is no assurance that ABP antibodies will penetrate the blood-testis barrier where ABP presumably works (25); indeed, studies by Setchell and his collaborators (103) have shown that macromolecules do not readily cross the blood-testis barrier.

LH RECEPTORS Numerous studies have demonstrated the presence of LH receptors in the interstitium of the testis (41, 42). These receptors, which undoubtedly are associated with Leydig cells, are probably responsible for the reception and transformation of an adenohypophyseal signal into the secretion of testosterone (43). Interference with the reception or transformation of the LH signal would result in a failure in testicular steroidogenesis and subsequently spermatogenesis. Apparently, hyperstimulation of the LH receptor on the Leydig cell renders it refractory to subsequent LH molecules and consequently reduces testosterone biosynthesis (44–46). Analogues of LRH have been identified (104, 105) that are markedly more potent than the naturally occurring LRH. One of these potent LRH analogues has been reported to inhibit gonadal function in male rats (106). It is possible that this treatment caused desensibilization of testicular LH receptors, with resultant diminution of steroidogenesis and spermatogenesis. Although this is an area worthy of research, one major shortcoming of such an antispermatogenic agent is that the androgen lack would result in a failure of libido and potency. Obviously, androgen substitution would be required.

INHIBIN The discovery of inhibin led to the speculation that it might be developed into an antispermatogenic agent which could be exploited as a male contraceptive (107). Theoretically, administration of inhibin to males would suppress FSH but not LH secretion. Supposedly, the unaltered LH would maintain testicular steroidogenesis and androgen-dependent peripheral functions while withdrawal of FSH would result in spermatogenic failure. While attractive on the surface, this theory suffers from shortcomings when critically analyzed. First, there is mounting evidence that FSH is required only for the initiation of spermatogenesis during puberty or after recovery from experimentally produced aspermatogenic states in a variety of species (53, 108, 109). Second, it has proven difficult to purify inhibin. While some investigators have partially purified the molecule (56, 63–67), others have found it difficult to reliably demonstrate its existence (110). Third, since inhibin is probably a proteinaceous molecule, when injected it should elicit antibodies neutralizing its biological activity. Fourth, it is now recognized that inhibin preparations also suppress LH as well as FSH secretion (56). This raises the possibility that treatment with inhibin might result not only in a decline in spermatogenesis but also in a decline in testicular steroidogenesis. Thus, even if we assume that treating adult males with inhibin will lower FSH secretion, that FSH is in fact required for spermatogenesis in the adult, and that a nonantigenic source of the material could be prepared, it is still likely that the individual would require androgen supplement to maintain libido and potency.

PINEAL PEPTIDES Pineal peptides offer two advantages over macromolecular antispermatogenic agents such as inhibin: (*a*) simple and inexpensive synthesis and (*b*) probable lack of antigenicity. Unfortunately, some features of these agents detract from their use as contraceptive agents. Since pineal peptides might normally exert their effect on hypothalamic nuclei via the cerebrospinal fluid, it could prove difficult to obtain high enough concentrations in peripheral circulation to achieve the desired effect. It is likely that pineal peptides would inhibit LH secretion which in turn would diminish both spermatogenesis and testicular steroidogenesis. Thus, as in the case of inhibin, and interference with LH receptors, androgen therapy would be required to maintain libido and potency.

Indoles

One could hypothesize that peripheral administration of melatonin, to man, would inhibit spermatogenesis in a manner analogous to that observed in the golden hamster and the grasshopper mouse (72). In adult man, however, too little information is presently available to state affirmatively whether indoles play, or have the capacity to play, any role in the control of spermatogenesis. Other limitations to the use of melatonin for male contraception are (*a*) that it is structurally analogous to neurotransmitters (i.e. serotonin) and therefore may have deleterious side effects and (*b*) that melatonin would probably decrease testicular steroidogenesis as well as spermatogenesis. Thus, once again androgen supplementation would be required.

Steroids

ANDROGENS Androgen treatment of males of several species is associated with two different effects on spermatogenesis depending on the dosage of androgen administered (111–114). At low doses, androgen suppresses gonadotropin secretion leading to decreased testicular steroidogenesis and hence decreased spermatogenesis. As androgen doses are increased, gonadotropin secretion remains depressed but the administered androgen now directly stimulates spermatogenesis. These observations are consistent with the hypothesis that a high concentration of androgen in the seminiferous tubule is essential for spermatogenesis (28, 115). Additional support for this hypothesis derives from the close physical proximity of the Leydig cells to the seminiferous tubules and the high concentration of testosterone in the testis and in rete testis fluid relative to that in peripheral blood (26, 27). Therefore, low doses of androgen administered peripherally (oral, intramuscular, or subcutaneous-sustained release) should lower intratesticular testosterone concentration, thus inhibiting spermatogenesis. However, the peripheral blood concentration of testosterone should be sufficient to maintain secondary sex characteristics.

The use of androgens as male contraceptive agents has been tested extensively in laboratory animals and man. The systematic search and selection for an appropriate dose that would inhibit gonadotropin secretion and spermatogenesis but be insufficient to maintain spermatogenesis on its own was first reported in 1973 (114, 116). When testosterone was administered to rabbits via sustained-release capsules [polydimethylsiloxane (PDS), Silastic®], azoospermia was attained while keeping peripheral testosterone level, libido, and accessory sex tissue weights normal.

Berndtson et al (28) and Reddy et al (117) have reported similar results using silastic capsules in the laboratory rat. Although some doses of testosterone were found to markedly diminish spermatogenesis in the rhesus monkey, no dose was found that caused azoospermia in all treated animals (118).

Though earlier reports of the suppression of spermatogenesis by testosterone in man exist (111, 113, 119, 120), the first positive test in humans of testosterone as a contraceptive agent was presented by Reddy & Rao in 1972 (121). Daily intramuscular injection of testosterone propionate (25 mg/day for 60 days) was found to induce azoospermia in all treated individuals. This inhibition in seven normal adult males was found to be entirely reversible and no deleterious side effects were observed during the treatment or recovery phases. Because of the impracticality of daily injections, a number of attempts have been made to administer testosterone in a depot form (testosterone enanthate) at time intervals of seven (122, 123), ten (124), fifteen (123), and thirty days (125) using doses of 200 or 250 mg. The results obtained by these different investigators were not entirely consistent; however, the general pattern that emerged was that intramuscular administration of 200 mg of testosterone enanthate every 10–14 days caused a marked suppression of spermatogenesis. Most recently, McClure et al (123) and Steinberger et al (124) confirmed in humans the observation previously made by Ewing and his collaborators in laboratory animals. (114, 116), namely, that a maximum decrease in spermatogenic activity can be obtained even when blood levels of testosterone remain within the normal range.

There are two major difficulties associated with injections of testosterone enanthate as a male contraceptive: (*a*) painful frequent administration and (*b*) inconsistent azoospermia. It is our opinion that both of these difficulties are easily surmountable. The first may be circumvented by administering the desired androgen, via subdermally placed androgen-filled sustained-release capsules. Such capsules could be designed to release steroids for protracted periods of time. The second objection may be overcome by either using an androgen that can more selectively inhibit gonadotropin release than stimulate spermatogenesis or by using a second steroid, in addition to testosterone, which could effectively decrease gonadotropin secretion without causing adverse effects. Progestins and estrogens (discussed below) are likely candidates for such steroids.

PROGESTINS Progestins are any compounds that induce the appropriate histological changes in the uterus of an experimentally prepared bioassay animal (126). Progestins that have been tested for their antispermatogenic properties fall into two general categories: (*a*) progesterone and its esters, (*b*) progestagens (synthetic progestins), which include 19-nortestosterone derivatives, (*c*) and antiandrogenic progestins.

Progesterone and its esters Progesterone has been reported to be weakly antispermatogenic in the rabbit (127) and ram (128), but totally ineffective in the rat (129). Similarly, 17α-hydroxy progesterone caproate inhibited spermatogenesis in man (130) but not in the rat (1). In general, the above compounds were found to inhibit

spermatogenesis poorly because of their weak antigonadotropic characteristics (2, 5). Additionally, progesterone and some of its metabolites can be converted to testosterone (131). Thus the weak antigonadotropic activity of these progestins may be further negated by their ability to act as substrates for testosterone synthesis.

Progestagens The 17-acetoxy derivatives of progesterone are generally poor antispermatogenic agents. However, the results are variable and confusing. Megestrol acetate (17α-acetoxy-6-methyl-Δ^6-progesterone) did not exert an antispermatogenic effect in rats (132). Algestone acetophenide (16α,17α-dihydroxy progesterone acetophenonide) suppressed spermatogenesis incompletely and unreliably (133). Medroxyprogesterone acetate (17α-acetoxy-6α-methyl-pregn-4-ene-3,20-dione) was thought to inhibit spermatogenesis without altering libido in the human (134); unfortunately, it was later reported that these results were not forthcoming in a second trial (5). The observation that these compounds are weakly antigonadotropic in rats and monkeys (2) probably accounts for the weak and variable antispermatogenic effects described above.

Some of the derivatives of androgens that are demethylated in the 19 position are progestational and have been found to be antispermatogenic in the rat and/or in man (135–141). These compounds include norethynodrel [17α-ethnyl-17β-hydroxy-5(10) estren-3-one], norgestrel (13β-ethyl-17α-ethynl-17β-hydroxy-4-estren-3-one), norethandrolone (17α-ethyl-17-hydroxy-4-nor androstan-3-one), norethindrone (17α-ethynl-17-hydroxy-4-nor-androsten-3-one), and ethynodiol diacetate (3β,17β-diacetoxy-17α-ethynyl-4-estrene). The antispermatogenic activity is probably caused by the potent antigonadotropic activity of these compounds (2). Consequently the antispermatogenic effect of these compounds is almost always accompanied by reduced libido and increased gynecomastia in man (130).

Cyproterone (6-chloro-17α-hydroxy-1α,2α-methylene-pregna-4,6-diene-3,20-dione) and cyproterone acetate have received considerable attention as antispermatogenic substances. Cyproterone, which has only weak antigonadotropic properties, was found to be a poor antispermatogenic agent (142). In contrast, cyproterone acetate, which inhibits gonadotropin secretion, was found to be an antispermatogenic agent (142). At doses of cyproterone acetate where inhibition of spermatogenesis was attained, marked diminution of accessory sex organ function in man (143), monkey (144), and rat (142) was also observed. The effects of cyproterone acetate on sexual behavior were not as clear. Some investigators (145, 146) claimed that it was possible to inhibit spermatogenesis in rats without affecting sexual behavior. However, this is not the case in man and subhuman primates (143, 144). Dosages of cyproterone acetate that completely inhibited spermatogenesis in these latter species also inhibited sexual behavior.

In summary, then, the antispermatogenic properties of progestagens are exceedingly variable and dependent on the amount and type of progestagen administered, its mode and period of administration, and, finally, the species of animal studied. Since antispermatogenic activity is well correlated with antigonadotropic activity, it is evident that simultaneous androgen therapy would be required to maintain secondary sexual characteristics.

ANDROGEN-PROGESTIN COMBINATIONS In 1973, Terner & MacLaughlin (147) reported the results of extensive tests of androgen-progestin combinations on spermatogenesis in rats. These investigators found that whereas medroxyprogesterone acetate and ethynodiol diacetate inhibited spermatogenesis and accessory sex organ growth, probably via their antigonadotropic effects, simultaneous treatment with carefully determined doses of testosterone maintained accessory sex organ size without restoring spermatogenesis.

When Coutinho & Melo (148), Frick (149), and Johansson & Nygren (150) tested similar combinations of androgens and progestins in the human male, the inhibition of spermatogenesis was variable and incomplete. Consequently, Frick and co-workers (151, 152) undertook an extensive evaluation of the androgen-progestin combinations on spermatogenesis. The drugs evaluated by these investigators included megestrol acetate, medroxyprogesterone acetate, norgestrienone (17α-ethynl-18-methyl norgestrienone), norethisterone (17α-ethynyl-estr-4-en-3-one), norethindrone, norethandrolone, testosterone, testosterone propionate, and testosterone enanthate. These drugs were administered to men of different ages in different combinations and via three different routes (oral, intramuscular, and subcutaneous-Silastic® implants). The shortcomings of these studies were that the treatment groups were small, sexual behavior was not critically evaluated, and spermatogenesis in older men was measured qualitatively via a histological examination of a testicular biopsy specimen. Therefore, we confine our remarks to experimental groups of young men with at least five individuals per treatment.

Though megestrol acetate or medroxyprogesterone acetate combined with androgens maintained libido and potency, these drugs failed to create consistent azoospermia (151, 152). The one treatment that combined a 19-nortestosterone derivative (norgestrieneone) with an androgen resulted in azoospermia in all treated males. Plasma concentrations of testosterone, LH, and FSH were depressed. Unfortunately, libido was suppressed and some men experienced deleterious side effects. The simultaneous administration of three drugs, norethisterone, norethindrone, and testosterone, once again resulted in variable antispermatogenic response. In summary, although no satisfactory androgen-progestin combination has been found that could be used as a male contraceptive, this approach is appealing and warrants further investigation.

DANAZOL, ANDROGEN-DANAZOL COMBINATIONS Danazol [17α-pregna-2,4-dien-20-yno(2,3-*d*)-isoxazol-17-ol] was reported to suppress gonadotropin secretion even though it had only weak androgen activity and had neither estrogen nor progestin activities (153). Administration of danazol to men caused a reduction of sperm count but not azoospermia. Plasma testosterone was diminished in these men but only minimal complaints of decreased libido were noted (154). More recent studies have shown that danazol plus testosterone propionate or testosterone enanthate caused a severe reduction in spermatogenesis in man (125, 155, 156). Thus, the addition of a testosterone ester apparently enhanced the inhibitory effect of danazol on spermatogenesis and overcame the problem of impaired libido and sexual potentia. This approach has not yet led to azoospermia in all individuals

tested, but is one of the combinations that may become acceptable when proper dosage and route of administration are elucidated.

ESTROGENS Estrogens have been recognized for more than thirty years as substances capable of suppressing testicular function (5, 130, 157, 158). In 1950, Ludwig (111) demonstrated that the histology of the testis after estrogen administration was comparable to that following hypophysectomy. It has since been shown that estrogens were highly effective inhibitors of gonadotropin release (50, 51). Thus, it has been hypothesized that estrogens exert their effect on the testis indirectly by suppression of LH and FSH release (50, 51, 159). In addition, it has been proposed by Steinberger & his collaborators (160) that high doses of estrogens may exert a direct effect on male reproductive tissues.

The administration of estrogens to laboratory animals (5, 157) and to humans (135) has resulted in azoospermia; however, the concomitant side effects such as decreased libido, potentia, and marked gynecomastia rendered these agents unacceptable in the doses used.

Though it was clear that a number of effects were associated with estrogen administration to males, there was no study whereby a spectrum of estrogen dosages were administered, preferably in a sustained-release manner, to determine whether the dose-response curve of effects such as gonadotropin suppression and gynecomastia could be separated. If such a situation were forthcoming, estrogen administration would have a place in male contraception. Alternatively, some of the unacceptable side effects associated with administration of antigonadotropic doses of estrogen probably could be countered by simultaneous androgen therapy.

ANDROGEN-ESTROGEN COMBINATIONS Briggs & Briggs (161) have suggested that the administration of combined androgen-estrogen formulations for the purpose of suppressing fertility in the male be investigated. In their pilot study (161), they found that daily oral administration of methyltestosterone and ethynylestradiol to normal adult men led to azoospermia in all five treated individuals. As was expected, this azoospermic state was associated with a lack of fertility. No sustained deleterious side effects were observed by this therapy and return to pretreatment content of spermatozoa in semen was attained within 35 weeks following cessation of treatment.

The essential need of further experimentation, using laboratory animals, in order to establish our understanding of the interaction between androgens and estrogens was apparent. Ewing and his collaborators (162) have recently demonstrated that in rats, a synergistic interaction between estradiol-17β and testosterone on the inhibition of spermatogenesis exists. Doses of testosterone or estradiol-17β (administered via Silastic® implants), which separately were ineffective in rendering the rats azoospermic, together brought about consistent azoospermia. The animals treated with such a steroid combination had plasma levels of testosterone and estradiol that were indistinguishable from those of the control animals. Moreover, wet weights of the seminal vesicle and the ventral prostate were found to be the same as those of untreated animals. No gynecomastia was detectable.

Though it is clear that more experimental work is still necessary, it is most promising that the administration of a combination of testosterone and estradiol-17β, both naturally occurring hormones in the male, may prove to be a safe, effective, and reversible male contraceptive when administered via a sustained-release mechanism.

CONCLUSION

This review clearly demonstrates that endogenous compounds exert regulatory influences at four major levels in the male: (*a*) between germ cells (e.g. spermatogonial chalone), (*b*) between Sertoli and germ cells (e.g. ABP), (*c*) between Leydig cells and seminiferous tubules (e.g. testosterone), and (*d*) between the central nervous system and the testis (e.g. the reciprocal relationship between LH secretion by the adenohypophysis and testicular steroid secretion).

To date, efforts to interrupt spermatogenesis have failed to find application as male contraceptives for various reasons: (*a*) some investigators ignored the vulnerable control points by utilizing nonspecific agents (e.g. antimitotic agents), (*b*) others attacked a vulnerable control point but used synthetic drugs that had deleterious side effects (e.g. orally active 17α-methyl androgens), and (*c*) still others attacked a vulnerable control point with a relatively innocuous drug (e.g. testosterone propionate) but used an impractical mode of drug administration (e.g. daily intramuscular injections).

In this review we have attempted to demonstrate the potential for devising innovative techniques (e.g. subcutaneous sustained release) for administering relatively innocuous drugs (e.g. gonadal steroids) at dosages sufficient to produce sterility without causing deleterious side effects.

The most promising immediate solution for the development of an antispermatogenic male contraceptive is the interference with the adenohypophyseal-gonadal axis via the subcutaneous sustained release of steroid formulations containing either androgen-danazol, androgen-progestin, or androgen-estrogen formulations. Another promising antispermatogenic agent that could be developed quickly would be an LRH agonist-androgen formulation. Requiring more research, but equally interesting, would be an antispermatogenic spermatogonial chalone.

ACKNOWLEDGMENT

This review was supported in part by the National Institute of Child Health and Human Development through Research Contract 3–2745, Research Grant 07204, and Population Center Grant 06268.

Literature Cited

1. Jackson, H., Jones, A. R. 1972. The effects of steroids and their antagonists on spermatogenesis. *Adv. Steroid Biochem. Pharmacol.* 3:167–92
2. Bennett, J. P. 1974. *Chemical Contraception.* pp. 133–70. New York: Columbia Univ. Press. 230 pp.
3. deKretser, D. M. 1974. The regulation of male fertility. The state of the art and future possibilities. *Contraception* 9:561–600
4. Neumann, F., Diallo, F. A., Hasan, S. H., Schenck, B., Traore, I. 1976. The influence of pharmaceutical compounds on male fertility. *Andrologia* 8:203–35
5. Patanelli, D. J. 1975. *Handb. Physiol., Sect. 7, Endocrinology* 12:245–58
6. deKretser, D. M. 1976. Towards a pill for men. *Proc. R. Soc. London Ser. B.* 195:161–74
7. Clermont, Y. 1972. Kinetics of spermatogenesis in mammals: Seminiferous epithelium cycle and spermatogonial renewal. *Physiol. Rev.* 52:198–236
8. Roosen-Runge, E. C. 1969. Comparative aspects of spermatogenesis. *Biol. Reprod.* 1:24–39
9. Fawcett, D. W. 1975. *Handb. Physiol., Sect. 7, Endocrinology* 2:21–51
10. Bullough, W. S., Laurence, E. B. 1960. The control of epidermal mitotic activity in the mouse. *Proc. R. Soc. London Ser. B.* 151:517–36
11. Houck, J. C. 1976. In *Chalones,* ed. J. C. Houck, 1:1–5. New York: Am. Elsevier. 510 pp.
12. Dym, M., Clermont, Y. 1970. Role of spermatogonia in the repair of the seminiferous epithelium following x-irradiation of the rat testis. *Am. J. Anat.* 128:265–82
13. Clermont, Y., Mauger, A. 1974. Existence of a spermatogonial chalone in the rat testis. *Cell Tissue Kinet.* 7:165–72
14. Clermont, Y., Mauger, A. 1976. Effect of a spermatogonial chalone on the growing rat testis. *Cell Tissue Kinet.* 9:99–104
15. McLaren, M. J. 1976. *Testicular chalones in the rat.* MS thesis. McGill Univ., Montreal, Canada. 90 pp.
16. Hansson, V. 1972. Further characterization of the 5α-dihydrotestosterone binding protein in the epididymal cytosol fraction. *In vitro* studies. *Steroids* 20:575–96
17. French, F. S., Ritzen, E. M. 1973. A high affinity androgen binding protein (ABP) in rat testis: Evidence for secretion into efferent duct fluid and absorption by epididymis. *Endocrinology* 95:88–93
18. Hansson, V., Trygstad, O., French, F. S., McLean, W. S., Smith, A. A., Tindall, D. J., Weddington, S. C., Petrusz, P., Nayfeh, S. N., Ritzen, E. M. 1974. Androgen transport and receptor mechanisms in testis and epididymis. *Nature* 250:387–91
19. Hansson, V., Ritzen, E. M., French, F. S., Nayfeh, S. N. 1975. *Handb. Physiology, Sect. 7, Endocrinology* 7:173–201
20. Tindall, D. J., Means, A. R. 1976. Concerning the hormonal regulation of androgen binding protein in rat testis. *Endocrinology* 99:809–18
21. Tindall, D. J., Hansson, V., McLean, W. S., Ritzen, E. M., Nayfeh, S. N., French, F. S. 1975. Androgen-binding proteins in rat epididymis: Properties of a cytoplasmic receptor for androgen similar to the androgen receptor in ventral prostate and different from androgen-binding protein (ABP). *Mol. Cell. Endocrinol.* 3:83–101
22. Bruchovsky, N., Wilson, J. D. 1968. The intranuclear binding of testosterone and 5α-androstan-17β-ol-3-one by rat prostate. *J. Biol. Chem.* 243: 5953–60
23. Fang, S., Liao, S. 1971. Androgen receptors. Steroid- and tissue-specific retention of a 17β-hydroxy-5α-androstan-3-one-protein complex by the cell nuclei of ventral prostate. *J. Biol. Chem.* 246:16–24
24. Hansson, V., Ritzen, E. M., French, F. S., Weddington, S. C., Nayfeh, S. N. 1975. Testicular androgen binding protein (ABP): Comparison of ABP in rabbit testis and epididymis with a similar androgen-binding protein (TeBG) in rabbit serum. *Mol. Cell. Endocrinol.* 3:1–20
25. Hansson, V., Ritzen, E. M., French, F. S. 1976. Androgen transport and receptor proteins in the testis. In *Human Semen and Fertility Regulation in Men,* ed. E. S. Hafez, pp. 51–61. Saint Louis: Mosby. 615 pp.
26. Ganjam, V. K., Amann, R. P. 1973. Testosterone and dihydrotestosterone concentration in the fluid milieu of spermatozoa in the repoductive tract of the bull. *Acta Endocrinol.* 74:186–200
27. Pujol, A., Bayard, F., Louvet, J. P., Boulard, C. 1976. Testosterone and dihydrotestosterone concentrations in

plasma, epididymal tissues, and seminal fluid of adult rats. *Endocrinology* 98:111–13

28. Berndtson, W. E., Desjardins, C., Ewing, L. L. 1974. Inhibition and maintenance of spermatogenesis in rats implanted with polydimethylsiloxane capsules containing various androgens. *J. Endocrinol.* 62:125–35
29. Steinberger, E., Root, A., Ficher, M., Smith, K. D. 1973. The role of androgens in the initiation of spermatogenesis in man. *J. Clin. Endocrinol. Metab.* 37:746–51
30. Tsang, W. N., Collins, P. M., Lacy, D. 1973. Steroid metabolism by the seminiferous tubules *in vitro* and spermatogenesis in the developing rat. *J. Reprod. Fertil.* 34:513–17
31. van der Molen, H. J., Grootegoed, J. A., deGreef-Bijleveld, M. J., Rommerts, F. F. G., van der Vusse, G. J. 1975. In *Hormonal Regulation of Spermatogenesis,* ed. F. S. French, V. Hansson, E. M. Ritzen, S. N. Nayfeh, 2:3–23. New York: Plenum. 526 pp.
32. Welsh, M. J., Wiebe, J. P. 1976. Sertoli cells from immature rats: *In vitro* stimulation of steroid metabolism by FSH. *Biochem. Biophys. Res. Commun.* 69:936–41
33. Ahmad, N., Haltmeyer, G. C., Eik-Nes, K. B. 1975. Maintenance of spermatogenesis with testosterone or dihydrotestosterone in hypophysectomized rats. *J. Reprod. Fertil.* 44:103–7
34. Dorrington, J. H., Armstrong, D. T. 1975. Follicle-stimulating hormone stimulates estradiol 17β synthesis in cultured Sertoli cells. *Proc. Natl. Acad. Sci. USA* 72:2677–81
35. Mulder, E., Brinkmann, A. O., Lamers-Stahlhofen, G. J. M., van der Molen, H. J. 1973. Binding of estradiol by the nuclear fraction of rat testis interstitial tissue. *FEBS Lett.* 31:131–36
36. Christensen, A. K., Mason, N. R. 1965. Comparative ability of seminiferous tubules and interstitial tissue of rat testes to synthesize androgens from progesterone-4-^{14}C *in vitro*. *Endocrinology* 76:646–56
37. Wilson, J. D. 1975. *Handb. Physiology, Sect. 7, Endocrinology* Chap. 25, pp. 491–508
38. Naftolin, F., Ryan, K. J., Davies, I. J., Reddy, V. V., Flores, F., Petro, Z., Kuhn, M. 1975. The formation of estrogens by central neuroendocrine tissues. *Recent Prog. Horm. Res.* 31:295–319
39. Styliano, M., Forchielli, E., Dorfman, R. I. 1961. The metabolism of 4-C^{14}-testosterone in rat testis homogenate. *J. Biol. Chem.* 236:1318–20
40. Bedrak, E., Samuels, L. T. 1969. Steroid biosynthesis by the equine testis. *Endocrinology* 85:1186–95
41. deKretser, D. M., Catt, K. J., Burger, H. G., Smith, G. C. 1969. Radioautographic studies on the localization of ^{125}I-labelled human luteinizing and growth hormone in immature rats. *J. Endocrinol.* 43:105–11
42. Catt, K. J., Dufau, M. L. 1973. In *Receptors for Reproductive Hormones,* ed. B. W. O'Malley, A. R. Means, pp. 379–418. New York: Plenum. 458 pp.
43. Mendelson, C., Dufau, M., Catt, K. J. 1975. Gonadotropin binding and stimulation of cyclic adenosine 3':5'-monophosphate and testosterone production in isolated Leydig cells. *J. Biol. Chem.* 250:8818–23
44. Sharpe, R. M. 1976. hCG-induced decrease in availability of rat testis receptors. *Nature* 264:644–46
45. Hsueh, A. J. W., Dufau, M. L., Catt, K. J. 1977. Gonadotropin-induced regulation of luteinizing hormone receptors and desensitization of testicular 3':5'-cyclic AMP and testosterone responses. *Proc. Natl. Acad. Sci. USA* 74:592–95
46. deFrance, H., Sanchez, P., Gallet, D., Saez, J. M. 1977. Desensibilisation par la gonadotrophine chorionic humaine (hCG): Mise en evidence d'un controle negatif des recepteurs des gonadotrophines par la hCG au niveau des cellules de Leydig du testicule. *C. R. Acad. Sci. Paris* 284:1429–32
47. Davidson, J. M., Bloch, G. J. 1969. Neuroendocrine aspects of male reproduction. *Biol. Reprod.* 1:67–92
48. Debeljuk, L., Arimura, A., Schally, A. V. 1972. Effect of testosterone and estradiol on the LH and FSH release induced by LH-releasing hormone (LH-RH) in intact male rats. *Endocrinology* 90:1578–81
49. Davidson, J. M., Damassa, D. A., Smith, E. R., Cheung, C. 1976. In *Regulatory Mechanisms of Male Reproductive Physiology,* ed. C. H. Spilman, T. J. Lobl, K. T. Kirton, pp. 151–68. New York: Am. Elsevier. 217 pp.
50. Santen, R. J. 1977. In *The Testis in Normal and Infertile Men.* ed. P. Troen, H. R. Nankin, pp. 197–211. New York. Raven. 578 pp.
51. Loreaux, D. L., Vigersky, R. A., Mary-

nick, S. P., Janick, J. J., Sherins, R.J. 1977. See Ref. 50, pp. 213–25
52. Means, A. R. 1976. See Ref. 49, pp. 87–98
53. Steinberger, E. 1971. Hormonal control of mammalian spermatogenesis. *Physiol. Rev.* 51:1–22
54. Mahesh, V. B., Muldoon, T. G., Eldridge, J. C., Korach, K. S. 1975. The role of steroid hormones in the regulation of gonadotropin secretion. *J. Steroid Biochem.* 6:1025–36
55. Swerdloff, R. S., Grover, P. K., Jacobs, H. S., Bain, J. 1973. Search for a substance which selectively inhibits FSH-effects of steroids and prostaglandins on serum FSH and LH levels. *Steroids* 21:703–22
56. Franchimont, P., Chari, S., Hazee-Hagelstein, M. T., Debruche, M. L., Duraiswami, S. 1977. See Ref. 50, pp. 253–70
57. Gomes, W. R., Hall, R. W., Jain, S. K., Boots, L. R. 1973. Serum gonadotropin and testosterone levels during loss and recovery of spermatogenesis in rats. *Endocrinology* 93:800–9
58. McCullagh, D. R. 1932. Dual endocrine activity of the testes. *Science* 76:19–20
59. Setchell, B. P., Jacks, F. 1974. Inhibin-like activity in rete testis fluid. *J. Endocrinol.* 62:675–76
60. Franchimont, P., Chari, S., Schellen, A. M. C. M., Demoulin, A. 1975. Relationship between gonadotrophins, spermatogenesis and seminal plasma. *J. Steroid Biochem.* 6:1037–41
61. Keogh, E. J., Lee, V. W. K., Rennie, G. C., Burger, H. G., Hudson, B., deKretser, D. M. 1976. Selective suppression of FSH by testicular extracts. *Endocrinology* 98:997–1004
62. DeJong, F. H., Sharpe, R. M. 1976. Evidence for inhibin-like activity in bovine follicular fluid. *Nature* 263:71–72
63. Baker, H. W. G., Bremner, W. J., Burger, H. G., deKretser, D. M., Dulmanis, A., Eddie, L., Hudson, B., Keogh, E. J., Lee, V. W. K., Rennie, G. C. 1976. Testicular control of FSH secretion. *Recent Prog. Horm. Res.* 32:429–69
64. Davies, R. V., Main, S. J., Young, M. G. W. L., Setchell, B. P. 1976. Bio-assay of inhibin-like activity in rete testis fluid and its partial purification. *J. Endocrinol.* 68:26P
65. Setchell, B. P., Davies, R. V., Main, S. J. 1977. In *The Testis,* ed. A. D. Johnson, W. R. Gomes, Chap. 6, IV: 189–238. New York: Academic. 637 pp.
66. Franchimont, P., Chari, S., Demoulin, A. 1975. Hypothalamus-pituitary-testis interaction. *J. Reprod. Fertil.* 44: 335–50
67. Franchimont, P., Chari, S., Hagelstein, M. T., Duraiswami, S. 1975. Existence of a follicle-stimulating hormone inhibiting factor "inhibin" in bull seminal plasma. *Nature* 257:402–4
68. Johnsen, S. G. 1964. Studies on the testicular hypophyseal feedback mechanism in man. *Acta Endocrinol. Copenhagen* 45: Suppl., 90, pp. 99–125
69. deKretser, D. M., Burger, H. G., Hudson, B. 1974. The relationship between germinal cells and serum FSH levels in males with infertility. *J. Clin. Endocrinol. Metab.* 38:787–93
70. Leonard, J. M., Leach, R. B., Couture, M., Paulsen, C. A. 1972. Plasma and urinary follicle-stimulating hormone levels in oligospermia. *J. Clin. Endocrinol. Metab.* 34:209–14
71. Steinberger, A., Steinberger, E. 1976. Secretion of an FSH-inhibiting factor by cultured Sertoli cells. *Endocrinology* 99:918–21
72. Turek, F. W., Desjardins, C., Menaker, M. 1976. Differential effects of melatonin on the testes of photoperiodic and nonphotoperiodic rodents. *Biol. Reprod.* 15:94–97
73. Reiter, R. J. 1976. In *Neuroendocrine Regulation of Fertility,* ed. T. C. Anand Kumar, pp. 215–26. Basel: Karger. 322 pp.
74. Kinson, G. A. 1976. In *Cellular Mechanisms Modulating Gonadal Action Advances in Sex Hormone Research,* ed. R. L. Singhal, J. A. Thomas, pp. 87–139. Baltimore: Univ. Park Press. 472 pp.
75. Hoffman, R. A., Reiter, R. J. 1965. Pineal gland: Influence on gonads of male hamsters. *Science* 148:1609–11
76. Reiter, R. J. 1969. Pineal function in long term blinded male and female golden hamsters. *Gen. Comp. Endocrinol.* 12:460–68
77. Desjardins, C., Ewing, L. L., Johnson, B. H. 1971. Effects of light deprivation upon the spermatogenic and steroidogenic elements of hamster testes. *Endocrinology* 89:791–800
78. Berndtson, W. E., Desjardins, C. 1974. Circulating LH and FSH levels and testicular function in hamsters during light deprivation and subsequent photoperiodic stimulation. *Endocrinology* 95:195–205

79. Martini, L., Fraschini, F., Motta, M. 1968. Peptide hormones. Neural control of anterior pituitary functions. *Recent Prog. Horm. Res.* 24:439–96
80. Tilstra, B., Prop, N. 1963. On the possible function of melatonin. *Acta Morphol. Neerl. Scand.* 5:289–94
81. Debeljuk, L. 1969. Effect of melatonin on the gonadotropic function of the male rat under constant illumination. *Endocrinology* 84:937–39
82. Kinson, G. A., Robinson, S. 1970. Gonadal function of immature male rats subjected to light restriction, melatonin administration, and removal of the pineal gland. *J. Endocrinol.* 47:391-92
83. Reiter, R. J. 1974. *Handb. Physiology, Sect. 7. Endocrinology* 41:519–50
84. Hoffmann, K. 1974. Testicular involution in short photoperiods inhibited by melatonin. *Naturwissenschaften* 61: 364–65
85. Reiter, R. J., Vaughan, M. K., Blask, D. E., Johnson, L. Y. 1974. Melatonin: Its inhibition of pineal antigonadotrophic activity in male hamsters. *Science* 185:1169–71
86. Reiter, R. J., Vaughan, M. K., Blask, D. E., Johnson, L. Y. 1975. Pineal methoxyindoles: New evidence concerning their function in the control of pineal-mediated changes in the reproductive physiology of male golden hamsters. *Endocrinology* 96:206–13
87. Turek, F. W., Desjardins, C., Menaker, M. 1975. Melatonin: Antigonadal and progonadal effects in male golden hamsters. *Science* 190:280–82
88. Ellis, L. C. 1972. Inhibition of rat testicular androgen synthesis in vitro by melatonin and serotonin. *Endocrinology* 90:17–28
89. Fraschini, F., Mess, B., Martini, L. 1968. Pineal gland, melatonin and the control of luteinizing hormone secretion. *Endocrinology* 82:919–24
90. Kamberi, I. A., Mical, R. S., Porter, J. C. 1970. Effect of anterior pituitary perfusion and intra-ventricular injection of catecholamines and indoleamines on LH release. *Endocrinology* 87:1–12
91. Martin, J. E., Engel, J. N., Klein, D. C. 1977. Inhibition of the *in vitro* pituitary response to luteinizing hormone-releasing hormone by melatonin, serotonin and 5-methyoxytryptamine. *Endocrinology* 100:675–80
92. Benson, B., Matthews, M. J., Rodin, A. E. 1971. A melatonin-free extract of bovine pineal with antigonadotrophic activity. *Life Sci.* 10:607–12
93. Benson, B., Matthews, M. J., Rodin, A. E. 1972. Studies on a nonmelatonin pineal antigonadotropin. *Acta Endocrinol.* 69:257–66
94. Orts, R. J., Benson, B. 1973. Inhibitory effects on serum and pituitary LH by a melatonin-free extract of bovine pineal gland. *Life Sci.* 12:513–19
95. Orts, R. J., Benson, B., Cook, B. F. 1974. Some antigonadotrophic effects of melatonin-free bovine pineal extracts in rats. *Acta Endocrinol.* 76:438–48
96. Orts, R. J., Kocan, K. M., Yonushonis, W. P. 1975. Fertility control in female rats by bovine pineal gland extracts. *Life Sci.* 17:531–38
97. Orts, R. J., Kocan, K. M., Wilson, I. B. 1975. Inhibitory action of melatonin on a pineal antigonadotropin. *Life Sci.* 17:845–50
98. Ebels, I., Benson, B., Matthews, M. J. 1973. Localization of a sheep pineal antigonadotropin. *Anal. Biochem.* 56: 546–65
99. Matthews, M. J., Benson, B. 1973. Inactivation of pineal antigonadotrophin by proteolytic enzymes. *J. Endocrinol.* 56:339–40
100. Benson, B., Matthews, M. J., Hruby, V. J. 1976. Characterization and effects of a bovine pineal antigonadotropic peptide. *Am. Zool.* 16:17–24
101. Rosenblum, I. Y., Benson, B., Hruby, V. J. 1976. Chemical differences between bovine pineal antigonadotropin and arginine vasotocin. *Life Sci.* 18: 1367–74
102. Orts, R. J. 1977. Reduction of serum LH and testosterone in male rats by a partially purified bovine pineal extract. *Biol. Reprod.* 16:249–54
103. Setchell, B. P., Waites, F. M. H. 1975. *Handb. Physiol.* Sect. 7. *Endocrinology* 6:143–72
104. Vale, W., Rivier, C., Brown, M., Leppaluoto, J., Ling, N., Monahan, M., Rivier, J. 1976. Pharmacology of hypothalamic regulatory peptides. *Clin. Endocrinol.* 5:S261–S73
105. Vale, W., Rivier, C., Brown, M. 1977. Regulatory peptides of the hypothalamus. *Ann. Rev. Physiol.* 39:493–527
106. Vale, W., Rivier, C., Rivier, J., Brown, M. 1977. Diverse roles of hypothalamic regulatory peptides. In *Proc. 5th Int. Symp. Med. Chem.* Amsterdam: Elsevier
107. Schoysman, R. 1976. Present state of

male contraception. *Spermatozoa Action Prog. Reprod. Biol.* 1:294–303
108. Lostroh, A. J. 1976. See Ref. 49, pp. 13–27
109. Madhwa Raj, H. G., Dym, M. 1976. The effects of selective withdrawal of FSH or LH on spermatogenesis in the immature rat. *Biol. Reprod.* 14:489–94
110. Braunstein, G. D., Swerdloff, R. S. 1977. See Ref. 50, pp. 281–91
111. Ludwig, D. J. 1950. The effect of androgen on spermatogenesis. *Endocrinology* 46:453–81
112. Walsh, P. C., Swerdloff, R. S. 1973. Biphasic effect of testosterone on spermatogenesis in the rat. *Invest. Urol.* 11:190–93
113. Nelson, W. O. 1937. Some factors involved in the control of the gametogenic and endocrine functions of the testis. *Cold Spring Harbor Symp. Quant. Biol.* 5:123–35
114. Ewing, L. L., Desjardins, C., Stratton, L. G. 1973. In *Temporal Aspects of Therapeutics,* ed. J. Urquhart, F. E. Yates, pp. 165–80. New York: Plenum. 213 pp.
115. Heller, C. G., Morse, H. C., Su, U., Rowley, M. J. 1970. In *The Human Testis,* ed. E. Rosenberg, C. A. Paulsen, pp. 249–57. New York: Plenum. 645 pp.
116. Ewing, L. L., Stratton, L. G., Desjardins, C. 1973. Effect of testosterone polydimethylsiloxane implants upon sperm production, libido and accessory sex organ function in rabbits. *J. Reprod. Fertil.* 35:245–53
117. Reddy, P. R. K., Prasad, M. R. N. 1973. Control of fertility in male rats by subcutaneously implanted silastic capsules containing testosterone. *Contraception* 7:105–14
118. Ewing, L. L., Schanbacher, B., Desjardins, C., Chaffee, V. 1976. The effect of subdermal testosterone filled polydimethylsiloxane implants on spermatogenesis in Rhesus monkeys (*Macaca mulata*). *Contraception* 13:583–96
119. Heller, C. G., Nelson, W. O., Hall, I. C., Henderson, E., Maddock, W. O., Jungck, E. C., Paulsen, C. A., Mortimer, G. E. 1950. Improvement in spermatogenesis following depression of human testes with testosterone. *Fertil. Steril.* 1:415–22
120. Heckel, N. J., McDonald, J. H. 1952. The effects of testosterone propionate upon the spermatogenic function of the human testis. *Ann. NY Acad. Sci.* 55: 725–33
121. Reddy, P. R. K., Rao, J. M. 1972. Reversible antifertility action of testosterone propionate in human males. *Contraception* 5:295–301
122. Mauss, J., Borsch, G., Richter, F., Bormacher, K. 1974. Investigations on the use of testosterone oenanthate as a male contraceptive. *Contraception* 10:281–89
123. McClure, R. D., Palacios, A., Brosman, S. A., Swerdloff, R. S. 1977. Suppression of spermatogenesis in normal subjects with testosterone enanthate (TE) — potential contraceptive agent. *Clin. Res.* 25:A149
124. Steinberger, E., Smith, K. D. 1977. Use of testosterone enanthate (TE) as a male contraceptive. *Fertil. Steril.* 28:290
125. Paulsen, C. A., Leonard, J. M. 1976. Clinical trials in reversible male contraception. I. Combination of Danazol plus testosterone. In *Regulatory Mechanisms of Male Reproductive Physiology,* ed. C. H. Spilman, pp. 197–211. Amsterdam: Elsevier & Excerpta Med. 217 pp.
126. Drill, V. A. 1959. Biological effects of some steroids with progestational activity. *Fed. Proc.* 18:1040–48
127. Ericsson, R. J., Dutt, R. H., Archdeacon, J. W. 1964. Progesterone as inhibitors of spermatogenesis in the rabbit. *Nature* 204:261–63
128. Ericsson, R. J., Dutt, R. H. 1965. Progesterone and 6α-methyl-17α-hydroxy progesterone acetate as inhibitors of spermatogenesis and accessory gland function in the ram. *Endocrinology* 77:203–8
129. Nelson, W. O., Patanelli, D. J. 1960. Inhibitory effects of steroids on gonadotrophin secretion in the male rat. *Acta Endocrinol.* Suppl. 51, p. 905
130. Heller, C. G., Moore, D. J., Paulsen, C. A., Nelson, W. O., Laidlaw, W. M. 1959. Effects of progesterone and synthetic progestins on the reproductive physiology of normal men. *Fed. Proc.* 18:1057–65
131. Nelson, W. O., Merckel, C. 1937. Maintenance of spermatogenesis in testis of the hypophysectomized rat with sterol derivatives. *Proc. Soc. Exp. Biol. Med.* 36:825–28
132. Karkun, J. N., Kar, A. B. 1965. Effect of megestrol acetate on the genital organs and fertility of male rat. *Indian J. Exp. Biol.* 3:213–15
133. Setty, B. S., Kar, A. B. 1966. Effects of Deladroxone on spermatogenesis in rats and rhesus monkeys. *Steroids* 8:33–43

134. MacLeod, J. 1965. In *Agents Affecting Fertility,* ed. C. R. Austin, J. S. Perry, pp. 93–123. Boston: Little, Brown. 319 pp.
135. Heller, C. G., Laidlaw, W. M., Harvey, H. T., Nelson, W. O. 1958. Effects of progestational compounds on the reproductive process of the human male. *Ann. NY Acad. Sci.* 71:649–65
136. Patanelli, D. J., Nelson, W. O. 1959. The effect of certain 19-nor steroids and related compounds on spermatogenesis in male rats. *Arch. Anat. Microsc. Morphol. Exp.* 48:199–222
137. Bose, A. R., Kar, A. B. 1965. Antitesticular effect of ethynodiol diacetate. *Endokrinologie* 49:55–64
138. Kar, A. B., Setty, B. S. 1965. Biological evaluation of cytopentyl enol ether of 17α ethynyl-19-nortestosterone acetate as a male oral contraceptive. *Indian J. Med. Res.* 53:1180–85
139. Setty, B. S., Kar, A. B. 1967. Interruption of spermatogenesis by percutaneous application of steroids. *Steroids* 10:687–98
140. Singh, J. N., Setty, B. S., Srivastava, K., Kar, A. B. 1972. Effect of norgestrel on spermatogenesis and fertility of rats. *Indian J. Exp. Biol.* 10:159–62
141. Brenner, P. F., Bernstein, G. S., Roy, S., Jecht, E. E., Mishell, D. R. 1975. Administration of norethandrolone and testosterone as a contraceptive agent for men. *Contraception* 11:193–207
142. Steinbeck, H., Mehring, M., Neumann, F. 1971. Comparison of the effects of cyproterone, cyproterone acetate and oestradiol on testicular function, accessory sexual glands and fertility in a long-term study on rats. *J. Reprod. Fertil.* 26:65–76
143. Morse, H. C., Leach, D. R., Rowley, M. J., Heller, C. G. 1973. Effect of cyproterone acetate on sperm concentration, seminal fluid volume, testicular cytology and level of plasma and urinary ICSH, FSH and testosterone in normal men. *J. Reprod. Fertil.* 32: 365–78
144. Michael, R. P., Plant, T. M., Wilson, M. I. 1973. Preliminary studies on the effect of cyproterone acetate on sexual activity and testicular function in adult male rhesus monkeys (*Macaca mulatta*). *Adv. Biosci.* 10:197–208
145. Zucker, I. 1966. Effects of an antiandrogen on the mating behavior of male guinea pigs and rats. *J. Endocrinol.* 35:209–10
146. Whalen, R. E., Edwards, D. A. 1969. Effects of the antiandrogen cyproterone acetate on mating behavior and seminal vesicle tissue in male rats. *Endocrinology* 84:155–56
147. Terner, C., MacLaughlin, J. 1973. Effects of sex hormones on germinal cells of the rat testis: A rationale for the use of progestin and androgen combinations in the control of male fertility. *J. Reprod. Fertil.* 32:453–64
148. Coutinho, E. M., Melo, J. F. 1973. Successful inhibition of spermatogenesis in man without loss of libido: A potential new approach to male contraception. *Contraception* 8:207–17
149. Frick, J. 1973. Control of spermatogenesis in men by combined administration of progestin and androgen. *Contraception* 8:191–206
150. Johansson, E. D. B., Nygren, K. G. 1973. Depression of plasma testosterone levels in men with norethindrone. *Contraception* 8:219–26
151. Frick, J., Bartsch, G., Marberger, H. 1974. Steroidal control of fertility in men. *Excerpta Med. Int. Congr. Ser.* 394:375–85
152. Frick, J., Bartsch, G., Marberger, H. 1976. Steroidal compounds (injectable and implants) affecting spermatogenesis in men. *J. Reprod. Fertil. Suppl.* 24:35–47
153. Potts, G. O., Beyler, A. L., Schane, H. P. 1974. Pituitary gonadotropin inhibitory activity of Danazol. *Fertil. Steril.* 25:367–72
154. Sherins, R. J., Gandy, H. M. Thorslund, T. W., Paulsen, C. A. 1971. Pituitary and testicular function studies. 1. Experience with a new gonadal inhibitor, 17α-pregn-4-en-20-yno(2,3-d) isoxazol-17-ol (Danazol). *J. Clin. Endocrinol. Metab.* 32:522–31
155. Skoglund, R. D., Paulsen, C. A. 1973. Danazol-testosterone combination: A potentially effective means for reversible male contraception. A preliminary report. *Contraception* 7:357–65
156. Ulstein, M., Netto, N., Leonard, J., Paulsen, C. A. 1975. Changes in sperm morphology in normal men treated with Danazol and testosterone. *Contraception* 12:437–44
157. Noble, R. L. 1938. Effect of synthetic oestrogenic substances on the body growth and endocrine organs of the rat. *Lancet* 2:192–95
158. Lacy, D., Lofts, B. 1965. Studies on the structure and function of the mammalian testis. 1. Cytological and histo-

chemical observations after continuous treatment with oestrogenic hormone and the effects of FSH and LH. *Proc. R. Soc. London Ser. B* 162:188–97
159. Swerdloff, R. S., Walsh, P. C. 1973. Testosterone and estradiol suppression of LH and FSH in adult male rats: Duration of castration, duration of treatment and combined treatment. *Acta Endocrinol. Copenhagen* 73:11–21
160. Chowdhury, M., Tcholakian, R., Steinberger, E. 1974. An unexpected effect of oestradiol-17β on luteinizing hormone and testosterone. *J. Endocrinol.* 60: 375–76
161. Briggs, M. H., Briggs, M. 1974. Oral contraceptive for men. *Nature* 252: 585–86
162. Ewing, L. L., Desjardins, C., Irby, D. C., Robaire, B. 1977. Synergistic interaction of testosterone and estradiol on the inhibition of spermatogenesis in rats. *Nature* 269:409–11