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The Functional Significance of FSH in Spermatogenesis and the Control of Its Secretion in Male Primates

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The aim of this review is to provide an integrative analysis of the role of FSH in the control of testicular function in higher primates, including man. Attention is focused on the action of FSH during neonatal development, puberty, and adulthood. Whether FSH is the major determinant of the adult complement of Sertoli cells and whether FSH is obligatory for the initiation, maintenance, and restoration of spermatogenesis is evaluated. The mechanism whereby the circulating concentration of FSH regulates spermatogonial proliferation to dictate the sperm production rate under physiological conditions in the adult is discussed in detail. Inhibin B is the major component of the testicular negative feedback signal governing FSHβ gene expression and FSH secretion, and the evidence for this view is presented. The review concludes with the presentation of a model for the operation of the FSH-inhibin B feedback control system regulating sperm production postpubertally in monkey and man, and with speculation on issues of clinical interest. (*Endocrine Reviews* 22: 764–786, 2001)

I. Introduction

THIS REVIEW WAS prompted, in part, by the original studies of FSH-deficient transgenic mice (1) and of men with inactivating mutations of the FSH receptor (2), indicating that FSH may not be an absolute requirement for male fertility. The unconditional acceptance of such a notion would have considerable impact on future directions of research taken to elucidate the cell biology underlying testicular function, and on strategies adopted either for treatment of male infertility or for the development of novel approaches for male contraception. Therefore, a critical evaluation of the role of FSH in the initiation and maintenance of testicular function in higher primates[1] is timely. This is particularly pertinent because spermatogenesis and its hormonal control appear to exhibit marked species differences. Most notably, although hypophysectomy of adult primates leads, as it does in rats, to regression of the seminiferous epithelium, in the former species only Sertoli cells and stem spermatogonia remain after removal of the pituitary, whereas in the rat spermatogenesis is arrested during spermiogenesis (4–7). It is unlikely that this species difference is due to incomplete ablation of the rodent pituitary, as originally suggested by Smith (8), because spermatogenesis is also arrested during spermiogenesis in rats chemically hypophysectomized with a GnRH receptor (GnRH-R) antagonist, which dramatically suppresses gonadotropin secretion (9–11). Thus, in primates the gonadotropic hormones are obligatory for development of differentiated spermatogonia, spermatocytes, and spermatids, whereas in rats it appears that a limited number of spermatids may be produced in the absence of a gonadotropin drive.

II. Site of FSH Action in the Testis

FSH action is exerted on cells expressing the FSH receptor (FSH-R), and the pathways underlying the transduction of a FSH signal have been recently reviewed in detail for this journal by others (12). In brief, the binding of FSH to its receptor results in a dissociation of the α-subunit of the receptor-associated Gs protein, which, in turn, leads to the activation of adenylyl cyclase and production of cAMP. cAMP releases the catalytic subunit of PKA, allowing for phosphorylation of numerous intracellular proteins including the transcriptional activator, cAMP response element binding protein. Other pathways for FSH signal transduction have been proposed, but the relative importance of these in mediating FSH action on the testis *in vivo* is unclear (12).

In the cynomolgus monkey (*Macaca fascicularis*), expression of the FSH-R has been found only in the testis (13), and with the exception of the observations of Orth and Christensen (14, 15), studies of several nonprimate species have

Abbreviations: Ad, Dark-type A; Ap, pale-type A; FSH-R, FSH receptor; GnRH-R, GnRH receptor; h, human; InhBP, inhibin binding protein; LH-R, LH receptor; MG, menopausal gonadotropin; r, recombinant.

[1] According to Romer (3), the higher primates, *Catarrhini*, are comprised of three families, namely *Cercopithecidae* (Old World monkeys), *Simiidae* (man-like apes) and *Hominidae* (human).

demonstrated that FSH binding in the testis is restricted to the Sertoli cell (see Ref. 12). The foregoing results have led to the widely accepted view that the Sertoli cell is the only target of FSH action in the male (12).

Sertoli cells are of central importance to the spermatogenic function of the testis for many reasons (16). These somatic cells maintain the cytoarchitecture of the germinal epithelium, produce nutrients that provide energy substrates to the germ cells and, in the primate, represent the only cellular component of the blood-testis barrier (17, 18). An important concept deriving from the cytoarchitectural and nutritional functions of the Sertoli cell is that each Sertoli cell can support the development of only a limited number of germ cells (19, 20). Thus, the complement of Sertoli cells in the adult testis dictates, in part, fertility because it is generally recognized that the density of sperm in an ejaculate is a significant parameter of fertility (21, 22). The latter dogma is supported by a recent retrospective study that reported that birth rate in a North American state (Minnesota) over a period of 24 yr fluctuated inversely with sperm density measured during this period in a population of 660 men who were being screened before vasectomy (23). It should be noted that the sperm counts of these men were in the range generally accepted to be fertile (21). In addition, the World Health Organization study of the contraceptive efficacy of T enanthate revealed that in those men rendered oligospermic by administration of the steroid ester, pregnancy rates were directly related to sperm concentration (24). For the foregoing considerations, the determinants of the number of Sertoli cells in the adult testis become important to understand.

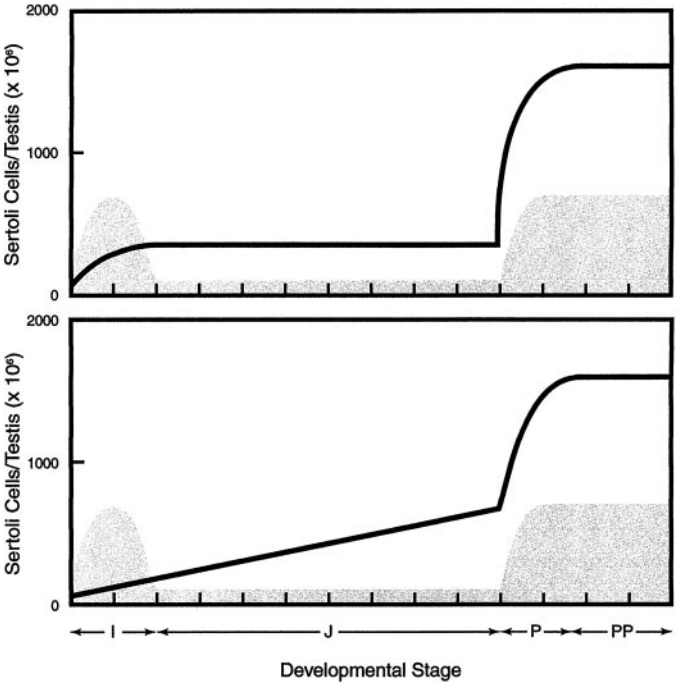

FIG. 1. Schemata of two hypothetical patterns of Sertoli cell proliferation during postnatal development in higher primates. For man, the ordinate would span 12–15 yr and for the monkey, 3–5 yr. The quantitative changes represented are based on data from the monkey (31) and may vary with species. The *upper panel* shows a biphasic mode of Sertoli cell proliferation with two distinct periods of mitosis: the first occurs during infantile development, and the second is initiated at the onset of puberty. In this model, Sertoli cell mitosis is dependent on the elevations in the secretion of both FSH and LH, which occur at infancy and again at puberty, as shown by the *shaded ghost*. The *lower panel* shows a pattern of Sertoli cell proliferation that is initially gonadotropin independent, occurs insidiously before the onset of puberty, and is followed, as in the first schemata, with a gonadotropin-dependent burst in Sertoli cell mitosis at puberty. I, Infant; J, juvenile; P, pubertal; PP, postpubertal.

III. Sertoli Cell Ontogeny and the Role of FSH

Sertoli cells first appear in the fetal human testis at approximately 8 wk of age (25), and at birth the testis of the infant contains approximately 10% of the adult complement of 4000 million cells (26). The findings that anencephaly in man (27) and fetal hypophysectomy in the rhesus monkey, *M. mulatta* (28), result at term in a marked reduction in testicular size suggests that gonadotropin secretion by the fetal pituitary plays an important role in regulating Sertoli cell proliferation *in utero*. The relative role of FSH in this regard is unclear, although the primate Sertoli cell expresses FSH-R early in development, as indicated by the finding of specific high affinity binding of radiolabeled FSH in homogenates of fetal testes from man and rhesus monkey at 8–16 and 19–22 wk gestation, respectively (29).

Sertoli cells continue to proliferate postnatally, as reflected by the finding that, in both monkey and man, the number of this cell type increases by a factor of 4–30 between infancy and adulthood (26, 30, 31). Although the temporal aspects of postnatal Sertoli cell proliferation have not been precisely described for any higher primate, two models may be proposed from the extant data (Fig. 1). A central feature of both schemata is a striking pubertal increase in Sertoli cell proliferation in association with the elevation in gonadotropin secretion that occurs at this stage of development. Two temporal patterns of Sertoli cell proliferation before puberty in higher primates may be proposed. The mitosis of Sertoli cells may occur throughout the entire prepubertal period (infantile and juvenile development), or alternatively, the prepubertal proliferation of this cell type may be restricted to infancy, a phase of development in Old World monkeys and man when gonadotropin secretion is elevated, as at the time of puberty (32).

Parenthetically, both of the posited patterns of Sertoli cell proliferation in higher primates might be considered to differ markedly from that in the rat, in which division of this cell type is generally accepted to be completed before puberty (20). This is because puberty in the male rat is usually recognized to be initiated between 25 and 30 d of age (20, 33), and Sertoli cell proliferation in this species is completed by 14–21 d of age (34). Differentiated spermatogonia (intermediate spermatogonia), however, are observed as early as 5–6 d of age in the rat (35), and if this developmental marker is used as the index of the onset of puberty, then the dogma that Sertoli cell proliferation is completed before puberty in this species would need to be reevaluated. In any event, whereas the adult complement of Sertoli cells in the rat is determined during the first few weeks of life, in higher primates this critical determinant of spermatogenic potential is not established until several years after birth. In the marmoset (*Cal-*

lithrix jacchus), a New World monkey, adult numbers of Sertoli cells are present at 18–22 wk of age (36), and in the Cebus monkey (*Cebus apella*), another New World primate, Sertoli cell proliferation is completed between 16 and 52 wk of age (37). Parenthetically, it may be noted here that because the adult complement of Sertoli cells in men is not attained until the second decade of life, the impact of endocrine disrupters on human fertility may be different from that in rapidly maturing species. Thus, comparative considerations need to be taken into account when selecting experimental models with which to examine the action of endocrine disrupters on the ontogeny of testicular function in men.

That Sertoli cell mitosis at the time of primate puberty is driven by the gonadotropic hormones is confirmed by the finding that proliferation of Sertoli cells may be induced precociously in juvenile rhesus monkeys, in which the pituitary-testicular axis is prematurely activated by intermittent stimulation with GnRH (31). FSH plays an important role in this process, as indicated by the findings that in the juvenile rhesus monkey, pulsatile stimulation with recombinant (r) human (h) FSH for 11 d resulted in a near doubling in Sertoli cell number in association with an increase in testicular size (38). This result is consistent with an earlier report of Nieschlag and his colleagues (39), who described an increase in Sertoli cell number per cross section of seminiferous cord after daily injections of purified hFSH to juvenile macaques. Although Sertoli cell number was not determined in the men described in 1997 with an inactivating mutation of the FSH-R gene, these individuals had small testicular volumes (2), also suggesting a role for FSH in the proliferation of this somatic cell type in men.

The pubertal proliferation of Sertoli cells in higher primates is probably not the consequence of an action of FSH alone, because pulsatile iv infusion of single-chain rhLH in juvenile rhesus monkeys for 11 days was as effective as rhFSH in stimulating Sertoli cell proliferation (38). Thus, it seems reasonable to propose that the gonadotropin milieu that provides the physiological stimulus for the initiation of the pubertal proliferation of Sertoli cells will be determined by the temporal relationship between the time courses of circulating FSH and LH concentrations during this developmental event. In the monkey, data describing pubertal changes in gonadotropin secretion are fragmentary, but in man an extensive literature exists on this subject. Although it was originally considered that, in boys, the increase in FSH secretion at the time of puberty preceded that of LH, application of more sensitive assays suggests that the pubertal activation of LH and FSH in the human male occurs concomitantly (32, 40, 41).

The action of LH to stimulate Sertoli cell proliferation is presumably mediated by a direct paracrine action of T secreted by the Leydig cell. This conclusion is supported by the finding that treatment of juvenile monkeys with T alone also stimulated division of this somatic cell type (39), and that primate Sertoli cells, like those of rodents, express the AR (42, 43). In this regard, it should be noted that the action of T to stimulate pituitary FSH secretion in GnRH-R antagonist-treated rodents (9, 10, 44) has not been observed in macaques (45). Additionally, the LH-induced proliferation of Sertoli cells in the juvenile monkey (38) occurs in the absence of an increase in plasma FSH concentrations (S. Ramaswamy, G. R. Marshall, and T. M. Plant, unpublished observations).

In higher primates, Sertoli cell proliferation ceases during puberty, and this stage of development is characterized by the appearance of mature or differentiated Sertoli cells, which typically exhibit a pleomorphic nucleus with a single distinctive nucleolus and specialized junctions that underlie the blood-testis barrier (17, 18). The mechanisms responsible for the differentiation of the primate Sertoli cell at the time of puberty are poorly understood. In juvenile macaques, administration of hCG, but not FSH, resulted in nuclear changes characteristic of adult Sertoli cells, and this was associated with a decline in the number of Sertoli cells expressing proliferating cell nuclear antigen (46). Moreover, because spermatogenesis is initiated in men with inactivating mutations of the FSH-R (2), it seems likely that Sertoli cell differentiation occurred in these subjects.

Before leaving the issue of Sertoli cell differentiation, the significance of the role of thyroid hormone in this regard merits discussion. Hypothyroidism in prepubertal boys may be associated with precocious enlargement of the testis in the absence of virilization (47), and this pathophysiological condition can lead to macroorchidism in adulthood (48). It seems reasonable to expect that the macroorchidism is associated with a greater than normal number of Sertoli cells because transient hypothyroidism induced in rat pups results in an increase in size of the adult testis and amplification of this cell type (49–51). Although the processes governing differentiation of the Sertoli cell appear to be arrested in the absence of thyroid hormone, those responsible for division are relatively unperturbed and as a result, the number of Sertoli cells is increased (50–51). Thus, the role of thyroid hormone in Sertoli cell differentiation, albeit obligatory, appears to be permissive, as is the action of this hormone in other tissues. Thus, it seems reasonable to conclude that Sertoli cell differentiation in higher primates is dictated by the pubertal rise in gonadotropin secretion.

IV. The Role of FSH in Spermatogenesis

A. Background

Before presenting a discussion of the role of FSH in governing spermatogenesis in higher primates, a brief review of this process, based on the work of others (52–57), will be presented. Spermatogenesis can be thought of as a process comprising three phases: stem cell renewal, germ cell proliferation, and spermiogenesis. Stem cell renewal is the mechanism that guarantees that a large and undiminishing number of undifferentiated germ cells are continually available for the subsequent steps of spermatogenesis. It is generally considered that there are two types of stem cells in higher primates: dark type A (Ad) and pale type A (Ap) spermatogonia.[2] The Ad spermatogonia divide rarely and are considered to provide a reserve population of stem cells. Although not extensively studied, Ad spermatogonia have been argued to play a greater role in stem cell renewal in man

[2] A third type of A spermatogonia, transitional A spermatogonia (At), has been described in macaques (57, 58).

(59–61). Whether this putative role of Ad spermatogonia involves division of this cell type has not been established. The Ap spermatogonia, however, are actively dividing stem cells and are therefore to be viewed as the renewing stem cells (62). Ap cells divide and produce two daughter Ap cells. Ten and one-half days later, in the case of the rhesus monkey (56), these daughter cells divide; 50% replicate themselves and the other half produce the first generation of differentiated or type B spermatogonia (type B_1), which marks the beginning of the proliferative phase of spermatogenesis.[3] In Old World monkeys, there are three mitotic divisions of the differentiated spermatogonia producing sequential generations of this cell type known as B_2, B_3, and B_4, respectively. This is followed by another mitotic division leading to the primary spermatocytes. Meiosis I and II results in the production of four haploid spermatids from each primary spermatocyte. Although not usually considered proliferation, meiosis also results in an increase in germ cell number and therefore contributes to and terminates the proliferative phase. In man, it has been reported that there is only one generation of B spermatogonia (59). Spermiogenesis, the last phase of spermatogenesis, comprises the morphological changes in the immature spermatids, which culminate in the generation of the highly differentiated testicular spermatozoa.

As shown in Fig. 2, there appear to be fundamental differences between the monkey and rat in the mechanism used for stem cell renewal and germ cell proliferation. According to Dym and Clermont (63), stem cell renewal in the rat involves sequential divisions to produce four generations of undifferentiated spermatogonia (A_1, A_2, A_3, and A_4), and therefore concomitantly contributes in a major fashion to germ cell proliferation.[4] In the monkey, on the other hand, stem cell renewal follows a far simpler scheme involving only one type of undifferentiated spermatogonia (type Ap),[2] and therefore contributes to proliferation only indirectly by the production of the first of the four generations of differentiated B spermatogonia (Fig. 2).

The production of testicular spermatozoa from undifferentiated spermatogonia is recognized to unfold according to a fixed kinetic program, with the cell cycle of each dividing germ cell and the morphogenic transformations during spermiogenesis considered to be immutable (52, 67).[5] As a result of this property of spermatogenesis, the germinal epithelium progresses through a species-dependent set of specific cellular associations referred to as stages of the seminiferous epithelial cycle. The duration of this cycle is constant for a particular species or breed. A schemata of the cycle of the seminiferous epithelium in the rhesus monkey is shown in Fig. 3. The design of this schemata emphasizes that the seminiferous epithelial cycle is continuous, that its duration is dictated by the 10.5-d cell cycle time of the undifferentiated type Ap spermatogonia (56), and that the number and composition of the specific cellular associations of the cycle are determined by the kinetics of the linear proliferation and differentiation of the progeny of the Ap spermatogonia.

Apoptosis is a normal component of spermatogenesis, and the maximal theoretical efficiency of producing testicular spermatozoa from undifferentiated spermatogonia is not reached (20, 70–72). In the rat, the majority of type A spermatogonia undergoes apoptosis (70), presumably due to the constraints imposed by the number of Sertoli cells in the rodent testis, and only 25–30% of the theoretical yield of differentiated spermatogonia is achieved (70). In the monkey, on the other hand, the Ap spermatogonia do not appear to undergo apoptosis because the number of this cell type remains constant across the cycle of the seminiferous epithelium (53). Moreover, in the monkey, all Ap spermatogonia divide each cycle of the seminiferous epithelium (53). Taken together, the latter two observations raise the possibility that, in nonhuman primates, the number of type Ap spermatogonia in the adult testis, rather than the number of Sertoli cells, determines the maximal spermatogenic capacity or ceiling of the testis.

If the number of Ap spermatogonia determines spermatogenic ceiling in higher primates, it would become important to understand the mechanisms whereby the adult complement of this cell type is attained. In this regard, the population of Ap spermatogonia in the adult monkey testis is an order of magnitude greater than that in the testis of the juvenile (31), and like the pubertal increase in Sertoli cells (see above), would appear to be gonadotropin dependent because precocious activation of FSH and LH secretion in the juvenile monkey by pulsatile GnRH treatment for 10 wk results in a 3-fold increase in the number of Ap spermatogonia (31). Moreover, because a shorter duration (11 d) of gonadotropic stimulation of the juvenile testis, achieved with recombinant preparations of human gonadotropin, failed to stimulate Ap spermatogonial number (38), it seems reasonable to propose that stem cell proliferation precedes in the wake of an expanding population of Sertoli cells.

B. Experimental paradigms

The issue of experimental models is important from a clinical standpoint because many aspects of the physiology and cell biology of the human testis are not amenable to study directly. When the differences described above in the spermatogenic process of rodents and primates are taken together with the relatively greater role of inhibin B in the testicular regulation of FSH secretion in the latter species (see *Section V*), it seems reasonable to conclude that a nonhuman primate may frequently represent the model of choice for integrative studies of testicular function and its control in men. It is now immediately necessary to recognize that there are differences in spermatogenesis between men and Old World monkeys, the family of nonhuman primates used most frequently as a paradigm for man. The full extent of these differences is probably not known at the present time, a situation resulting in large part from the difficulty encoun-

[3] The temporal sequence of stem cell renewal in the Old World monkeys varies among species, and in man the mechanism of this process is not entirely clear (52, 53, 57).

[4] An alternative mechanism of stem cell renewal in the rat has been proposed (64, 65), and an evaluation of both models was recently reported (66). Although de Rooij and Russell (66) argue that the Huckins' schemata of stem cell renewal in the rat is applicable to primates, a discussion of this view falls beyond the scope of the present chapter, the focus of which is on the action of FSH to amplify the population of differentiated B spermatogonia.

[5] There is evidence that the kinetics of spermatogenesis in immature rodents differ from that in adult rodents (68, 69).

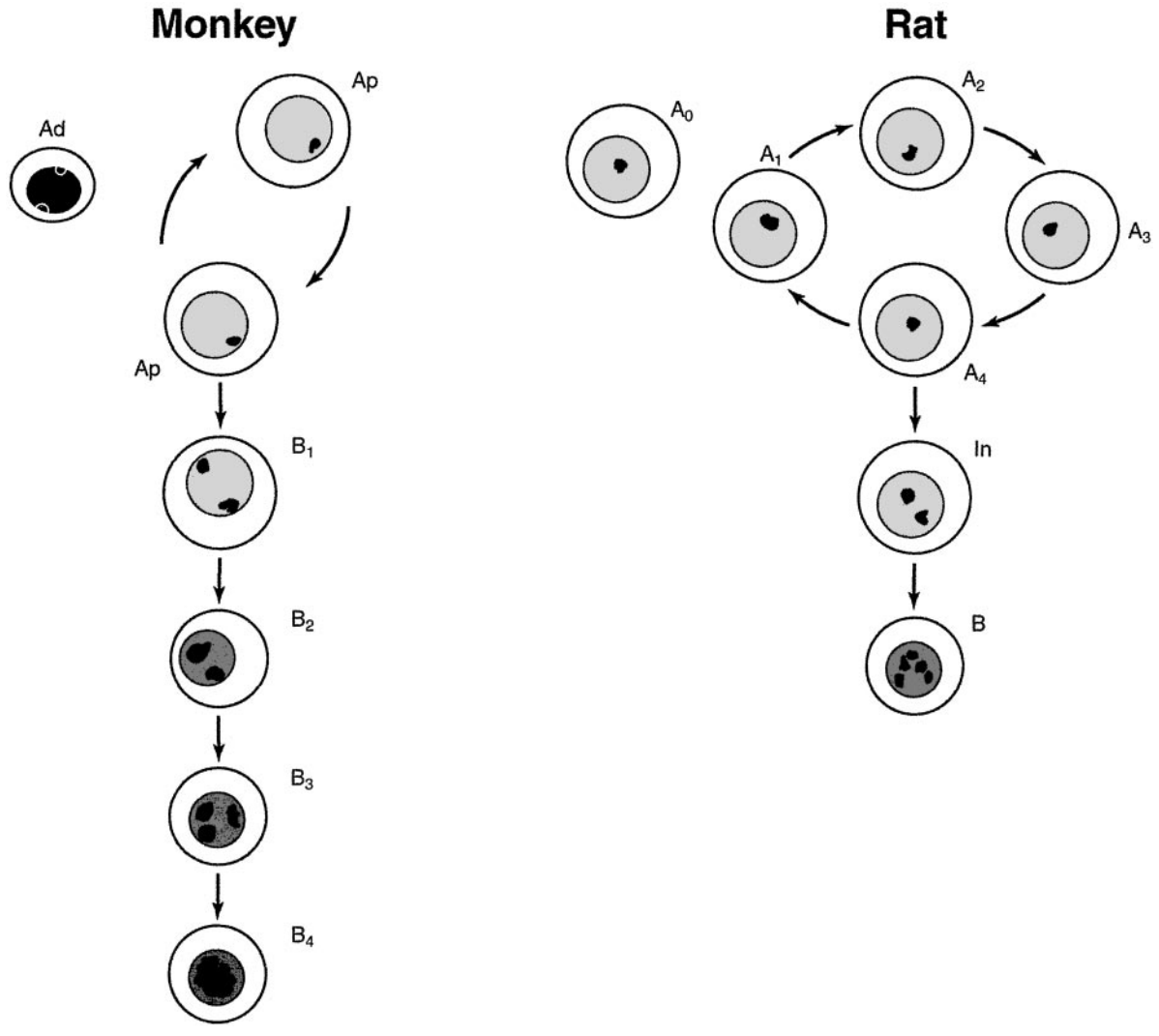

FIG. 2. Schematic patterns of stem cell renewal and spermatogonial proliferation in the monkey (*left*) and rat (*right*). In the monkey, Ap spermatogonia are the renewing stem cells, and the major phase of germ cell proliferation results from mitosis of differentiated type B_1 to B_4 spermatogonia. In rat, type A_1–A_4 spermatogonia are the renewing stem cells, and mitosis of these cells constitutes the major phase of germ cell proliferation. The last division of the renewing stem cells (A_4) in rat leads to the first of two generations of differentiated spermatogonia [intermediate (IN) and B]. It should be noted that the schematic representation of the division of Ap in monkey and of A_4 in rat does not necessarily imply asymmetric mitosis. The reserve stem cells, termed Ad spermatogonia in primates and A_o spermatogonia in rats, rarely divide. *Arrows* indicate mitosis. Derived from Refs. 53 and 63. See also footnotes 2 and 4.

tered in studying the human testis. Fundamental studies of human spermatogenesis and its kinetics involving the local injection of tritiated thymidine into ligated areas of the testis were conducted in a limited number of men in the 1960s (59). These early studies have not been replicated for ethical reasons, and inevitably, the data at hand are scant. Nevertheless, those differences that have been established merit identification. Perhaps the most notable difference between these primates relates to the number of generations of differentiated B spermatogonia (four in the monkey and one in man). Also, cross sections of the seminiferous tubule in the monkey exhibit only a single stage of the seminiferous epithelial cycle, but in man multiple stages are observed (59). Parenthetically, this cytoarchitectural arrangement of the human seminiferous tubule contributes to the difficulties encountered when estimating durations of the seminiferous epithelial cycle and of spermatogenesis. Other differences include the duration of spermatogenesis, which in the monkey is 48 d (56), whereas that for man has been reported to vary around 74 d (59), as well as the lesser number of stages of the seminiferous epithelial cycle in man (59). The latter presumably reflects species differences in either cell cycle time or in the duration of spermiogenesis and does not imply necessarily distinct endocrine or paracrine control systems governing the germinal epithelium in monkey and man. In any event, these differences, and the uncertainty of the precise nature of key spermatogenic steps in men, such as the function of Ad spermatogonia, must be borne in mind in the extrapolation of results obtained in the monkey to the human situation.

Moreover, as with any experimental model, the use of the monkey for studying human testicular function and its control has limitations, and consideration of some general ca-

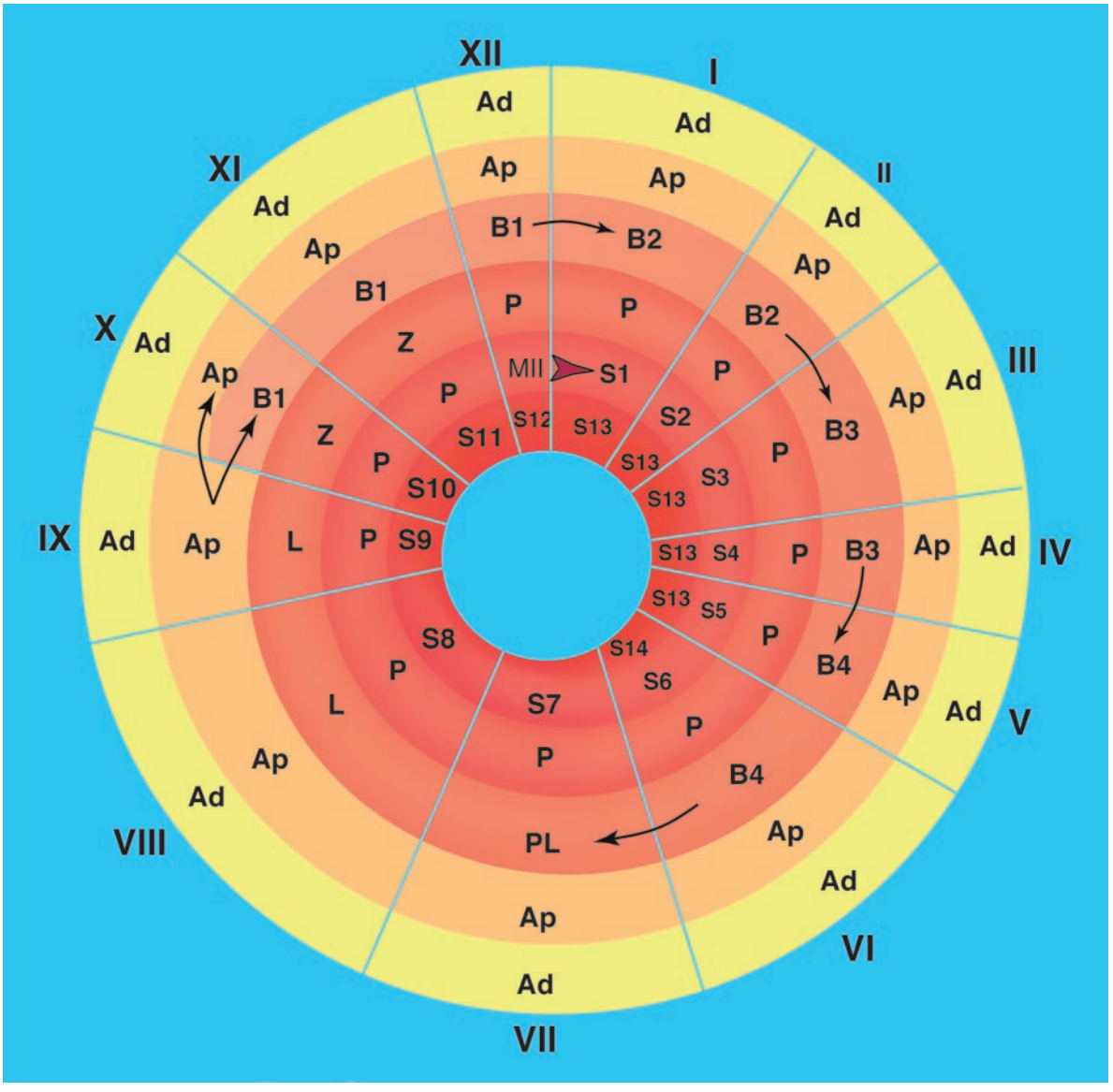

FIG. 3. The cycle of the seminiferous epithelium of the rhesus monkey depicted in a highly schematic manner to emphasize its recurring nature. The cellular associations that define the stages of the cycle as described by Clermont (55) are indicated with *Roman numerals I–XII*. The *circumference* represents time, and the *width of each segment* is proportional to the duration of each stage, which was derived from the frequency of each stage in 1000–2000 seminiferous tubular cross sections from each of 4 adult male rhesus monkeys (G. R. Marshall, unpublished observations). The increasing intensity of the *orange/red color* represents maturation of the cell types as the process of spermatogenesis unfolds. Spermatogenesis begins in stage IX when half of the population of Ap spermatogonia divide and produce the first generation of type B spermatogonia (B1). Coincidentally, the remaining Ap spermatogonia also divide to produce Ap spermatogonia, thereby renewing the population of these stem cells. Spermatogenesis terminates in stage VI when S14 spermatids are released into the lumen of the seminiferous tubule. B1–B4, Four generations of type B spermatogonia; PL, preleptotene primary spermatocytes; L, leptotene spermatocytes; Z, zygotene spermatocytes; P, pachytene spermatocytes; MII, completion of meiosis; S1–S14, spermatids at each of the 14 steps of spermiogenesis. The *arrows* and *arrowhead* indicate mitosis and the completion of meiosis, respectively. A similar approach to the representation of mammalian spermatogenesis was previously employed by Roosen-Runge (72).

veats is relevant here. In many species of monkey, and particularly in the rhesus macaque, the endocrine and spermatogenic activities of the testis, under feral conditions, exhibits marked seasonal fluctuations (74). Although such fluctuations are markedly attenuated or abolished under the rigidly controlled environmental conditions of the laboratory, information on the timing of experiments with respect to the breeding (fall-winter) or nonbreeding (summer) season is nevertheless necessary to fully interpret results.

Although recombinant macaque and baboon FSH and LH have recently been made available to investigators by the National Hormone and Pituitary Program, these remain in such limited supply that *in vivo* experiments requiring administration of homologous gonadotropins for several weeks are generally not possible. Human preparations must be used for such investigations, and use of heterologous gonadotropins *in vivo* raises the possibility that circulating antigonadotropin antibodies will be generated in the treated

monkeys (75), and therefore, negative results in such studies must always be interpreted with caution.

Limitations common to rodent models apply equally well to primates. Neither surgical nor chemical hypophysectomy is ideal. The former leads to loss of all pituitary hormones, and the possibility exists that systemic administration of GnRH and its analogs may exert direct actions on the primate testis in view of the reports that GnRH-R is expressed in this tissue (76–78). The ability to establish that gonadotropin secretion has been abolished in hypogonadotropic models rests on the sensitivity and specificity of the assays employed to assess circulating FSH and LH levels. In this regard, it is to be noted that the sensitivities of the RIAs that are available for measuring the macaque pituitary gonadotropins are probably markedly less than those of commercially available immunofluorometric assays for measuring circulating FSH and LH in men.

The number of sperm in an ejaculate is an extremely variable parameter, and therefore caution should be exercised when using sperm number or sperm concentration as a quantitative index of spermatogenesis. Additionally, the use of testicular biopsies for quantitative analysis of spermatogenesis must be interpreted conservatively because the limited amount of tissue harvested may not be representative of the entire organ. Serial biopsies from the same testes present an additional problem because the gonad may be damaged by the surgical procedure. Interpretation of the results obtained by studies employing passive or active immunoneutralization of circulating gonadotropin may not be straightforward. With negative data, the degree to which the circulating gonadotropin was inactivated is difficult to assess, and in experiments in which immunoneutralization interferes with spermatogenesis, nonspecific effects of the immunoneutralization procedure need to be evaluated with the inclusion of appropriate control experiments. These latter caveats apply equally well to studies of both the human and nonhuman primate.

C. Initiation of spermatogenesis

Initiation of spermatogenesis, which occurs at the time of puberty and, in higher primates, is associated with the transition from a relatively hypogonadotropic state of the prepubertal phase of development to the eugonadotropic state of adulthood (32), may be defined as the process that leads to the development of the first generation of testicular spermatozoa. Spermatogenesis may be initiated precociously in higher primates when FSH and LH secretion are prematurely elevated either experimentally (79) or pathophysiologically (80), establishing that the gonadotropic hormones provide the principal drive for this process. In patients with hypogonadotropic hypogonadism, hCG in combination with human menopausal gonadotropin (hMG) or FSH represents the treatment of choice for initiating spermatogenesis (81–84). Therefore, in the context of the present review, the question becomes, "What is the relative importance of FSH in this process?" A classical approach to examine this issue has been to provide the quiescent prepubertal primate testis with selective FSH stimulation, and to determine whether spermatogenesis is initiated with such a monotropic drive. Up to 12 wk of administration of hFSH alone to the prepubertal monkey resulted in a stimulation of the germinal epithelium, although cells more mature than B spermatogonia were not observed, indicating that a selective increase in FSH stimulation is unable to initiate spermatogenesis (38, 39, 46). This notion is reinforced by the finding that pulsatile stimulation of the testes in two juvenile monkeys with recombinant cynomolgus FSH for 18 wk resulted in germ cells no more mature than differentiated spermatogonia (S. Ramaswamy, G. Marshall, and T. Plant, unpublished observations). Here, it is interesting to note that in men with hypogonadotropic hypogonadism, azoospermia persisted during 2 yr of combined treatment with FSH and T (83). Presumably, the dose of T employed (250 mg T enanthate/week) was insufficient to restore normal intratesticular T content, and therefore, these men provide a clinical paradigm of selective FSH stimulation.

Hypogonadism resulting from inactivating mutations of the LH receptor (LH-R) provides an additional paradigm of selective FSH stimulation, although the abdominal or inguinal location of the testis in such subjects confounds the interpretation of these clinical data. In such patients, spermatogenic arrest has been consistently observed, occurring as early as the first meiotic division or as late as spermatid elongation (85, 86). In cases of complete androgen insensitivity, spermatogenesis is not initiated and the only germ cells observed in the testis are spermatogonia (87). Again, however, the testes are abdominal. A discussion of the fertile eunuch is merited here, because the syndrome was originally considered to reflect a selective loss of LH (88). Subsequent studies, however, have revealed that these men have low to low-normal LH levels in association with circulating T concentrations that are higher than those seen in infertile patients with idiopathic hypogonadotropic hypogonadism (89), and as the latter authors suggest, spermatogenesis in these subjects may be initiated by the combined action of intratesticular T, albeit at a reduced level, and relatively normal circulating FSH concentrations.

The inverse approach to examining the role of FSH in initiating spermatogenesis is to determine whether the process may be imposed prematurely in the juvenile, in the absence of this gonadotropin. In the juvenile macaque, T administration for 3 months stimulated the germinal epithelium, resulting in the premature appearance of primary spermatocytes (39), and exposure to T for 12 months initiated spermatogenesis while the animals were at a prepubertal age (90). The foregoing experimental observations in the monkey are consistent with reports of precocious initiation of spermatogenesis in boys with activating mutations of the LH-R (91) and Leydig cell hyperplasia (92, 93). They are also in keeping with the finding that hCG treatment, alone, initiates spermatogenesis in men with hypogonadotropic hypogonadism (81, 94).

Individuals with isolated FSH deficiency of unknown etiology, and with an apparently otherwise normal phenotype, provided the initial opportunity to examine whether spermatogenesis in the human male is initiated with LH alone at the time of spontaneous puberty (95–99). The seven men described in the foregoing studies, however, should be viewed as FSH insufficient rather than FSH deficient, and

therefore, the significance of the oligospermia observed in the majority of patients is difficult to evaluate.

The recent reports of men with a mutation in the gene encoding either the FSH-R (2) or the FSH β-subunit (100, 101) have provided additional and potentially cleaner paradigms of FSH deficiency. In all five subjects with the inactivating mutation of the FSH-R, spermatogenesis, as reflected by semen analysis, had been initiated (2). A similar phenotype was subsequently reported for transgenic FSH-R-knockout mice (102). The cell biology of the mutated human receptor, however, merits some discussion. Immortalized mouse Sertoli cells (MSC-1) transfected with the mutant FSH-R gene exhibited a comparable binding affinity but dramatically reduced FSH binding capacity and FSH-induced cAMP production when compared with MSC-1 cells expressing the wild-type FSH-R (103). The compromised signal transduction by the mutated receptor gene in MSC-1 cells may be accounted for, in part, by the apparent 30-fold lower incorporation of the mutant receptor into the membrane (103), perhaps resulting from a defect in the cellular trafficking of the mutant receptor. The absolute levels of expression of the mutated FSH-R within the testes of the affected patients, however, was not established, and because reduced FSH signaling up-regulates its own receptor (104, 105), the possibility that the mutated receptor may be overexpressed in these men should not be excluded. In addition, circulating FSH concentrations in the affected men were substantially higher than those in normal subjects. Hence, with the available information, it is not possible to conclude unequivocally that the FSH drive to the Sertoli cells in these subjects was completely abolished.

The foregoing concern is reinforced by the finding that, in contrast to the men with the FSH-R mutation, azoospermia and infertility were consistent features of the two men described with FSH deficiency due to deletion of the gene encoding the β-subunit of this gonadotropin (95, 101). Theoretically, the absence of FSH or the inactivation of its receptor should have the same impact upon testicular function, as observed in transgenic mice deficient in either FSHβ (1) or FSH-R (102). Thus, the difference in phenotype in these two groups of men is paradoxical and needs to be reconciled. The unsuccessful treatment with rhFSH of one of the men with the FSHβ mutation (106) does not necessarily imply an additional deficit in FSH signaling in this individual: the initial attempt to restore ovulation with hMG in a woman with isolated FSH deficiency was also unsuccessful due to the production of anti-FSH antibodies in response to hMG (107). The other FSH-deficient man had low serum T levels, and the relationship between the hypoandrogenism and the FSHβ mutation, if there is one, is not known (100). It should be noted that these interesting receptor mutations in men have also been recently discussed by Themmen and Huhtaniemi (108).

In accord with classical studies (see Ref. 109), an interaction between the two gonadotropins to initiate primate spermatogenesis is suggested by the recent finding that although intermittent stimulation of juvenile male monkeys with either LH or FSH alone for 11 d failed to produce germ cells more mature than differentiated type B_1 spermatogonia, the testis of monkeys receiving combined pulsatile gonadotropin treatment for a similar period showed the presence of all four generations of differentiated spermatogonia (B_1, B_2, B_3, and B_4) and preleptotene and leptotene-zygotene spermatocytes (38). The cell biology underlying the synergism between LH and FSH to initiate spermatogenesis is unclear. We propose, however, that because chronic exposure of the prepubertal primate testis to T eventually initiates spermatogenesis (see above), combined gonadotropin stimulation of the undifferentiated Sertoli cell in the juvenile minimizes the time required for maturation of this cell type. Presumably, FSH facilitates the posited LH-dependent differentiation of Sertoli cells (see above). Thus, the time taken for the Sertoli cell to acquire the potential to support spermatogenesis would be reduced under conditions of combined stimulation. In the context of the foregoing hypothesis, it is important to note that the kinetics of spermatogenesis would be identical regardless of whether spermatogenesis is initiated with either LH alone or with LH and FSH in combination.

In summary, the evidence to date supports the view that FSH may not be required for the initiation of spermatogenesis in primates, although this conclusion must be tempered with the caveat raised by the differing phenotypes exhibited by men with mutations of the FSH-R and the FSH β-subunit. At the same time, however, it should be recognized that the foregoing view does not necessarily imply that LH is obligatory for the initiation of spermatogenesis. This must await the confirmation that this developmental event is not elicited by a selective and sustained pulsatile FSH stimulation of the juvenile testis.

D. Maintenance of spermatogenesis

The maintenance of spermatogenesis, which has been recently reviewed by others (110, 111), may be defined as the process that leads to the sustained production of testicular spermatozoa in the adult testis; the number of these germ cells may be low or high. This notion of the maintenance of spermatogenesis corresponds to what has been previously called "qualitative maintenance" by others (112). It should be noted that the level at which spermatogenesis is maintained in higher primates is not invariant, but rather is subjected to regulation by physiological cues. For example, in certain species of monkey, particularly the rhesus macaque (74), and also in man, sperm count exhibits seasonal fluctuations with a decrease in sperm output during the summer months (113, 114). In primates, however, azoospermia probably does not occur during the nonbreeding season, and therefore, the seasonal paradigm should not be employed as a model for the restoration of spermatogenesis.

If, as argued above, the initiation of spermatogenesis requires only LH stimulation, then it may be inferred that the maintenance of spermatogenesis will also be driven by LH alone. Indeed, a considerable body of evidence exists to suggest that FSH is not obligatory for the maintenance of spermatogenesis in primates. In macaques, surgical or chemical hypophysectomy with immediate T replacement fails to interrupt the maintenance of spermatogenesis (115, 116). Similarly, spermatogenesis is also maintained in normal men rendered FSH deficient during hCG administration (117),

and by hCG treatment alone in patients with hypogonadotropic hypogonadism (81, 94).

Moreover, there have been many studies of the impact of immunoneutralizing circulating FSH concentrations in bonnet (*M. radiata*) and rhesus macaques, and in none of these has the maintenance of spermatogenesis been consistently interrupted (118–122). Additionally, in a single study of five human subjects actively immunized with ovine FSH over a 10-wk period (123), azoospermia was not observed. Lastly, a reduction in FSH drive to the testis of the adult bonnet monkey, produced by immunoneutralization against the FSH-R (124), did not abolish spermatogenesis, and men with an inactivating mutation of the FSH-R have sperm in semen and can be fertile (2). In view of the substantial body of evidence in support of the notion that LH alone can maintain spermatogenesis in the monkey, the recent observation that severe oligospermia (mean sperm number, $<0.2 \times 10^6$/ejaculate) or azoospermia was induced in the apparent face of normal intratesticular T concentrations in cynomolgus monkeys rendered hypogonadotropic with im T buciclate injections (125) is surprising. Perhaps an explanation for this finding might be that testicular T content, which was determined 8 wk after the last injection of the steroid, did not reflect androgen status of the testis at wk 5, 6, and 7, the phase of the experiment when the spermatogenic activity of the testis would have been reflected in the ejaculates collected at wk 6, 7, and 8 (126).

The question of whether LH is obligatory for the maintenance of spermatogenesis remains to be unequivocally addressed. In the bonnet macaque, active immunoneutralization of circulating LH with ovine LH for 43 wk resulted in a profound depletion in spermatocytes and spermatids (127), underlining the critical importance of this gonadotropin for spermatogenesis. In the cynomolgus monkey, the number of elongated spermatids was also greatly reduced after 8 wk of LH deficiency, achieved by concomitant GnRH-R antagonist treatment and FSH replacement (128). However, a more protracted study of the LH-deficient condition is ideally needed to determine whether FSH alone is incapable of maintaining spermatogenesis in nonhuman primates. The clinical studies pertaining to this issue are also equivocal. In hypogonadotropic hypogonadal men in which spermatogenesis had been initiated with hMG and hCG, substituting FSH and T for the original treatment failed to maintain spermatogenesis, as reflected by the finding that the patients became azoospermic (83). In a hypophysectomized man expressing a mildly activating mutation/polymorphism of the FSH-R (129), spermatogenesis was found to be maintained after withdrawal of T replacement. The maintenance of spermatogenesis in this individual, however, may not have been maintained by an FSH drive alone because circulating T levels declined to only 5.4 nmol/liter after androgen withdrawal (129).

Although it may be concluded that FSH stimulation is probably not obligatory for the maintenance of spermatogenesis in higher primates, it is premature to infer that LH is sufficient and necessary. Indeed, this will require the development of experimental paradigms in which LH drive may be selectively abolished while chronically preserving a physiological pulsatile FSH drive to the testis.

E. Restoration of spermatogenesis

Although the adult testis, in which regression of the seminiferous tubule has been imposed in response to experimentally induced hypogonadotropism, has been used as a model with which to examine the initiation of spermatogenesis, the processes of initiation and restoration exhibit several salient differences. First, maturation of the germinal epithelium at the time of initiation is intimately associated with Sertoli cell proliferation and differentiation, whereas during restoration, the germinal epithelium matures in the presence of an invariant number of differentiated Sertoli cells. Second, Sertoli cell function is determined in part by the composition of the basement membrane of the seminiferous tubules (130), which exhibits developmental changes (131, 132), and these changes may not be recapitulated during restoration of spermatogenesis of a regressed adult testis. Although the validity of using restoration of spermatogenesis as a paradigm for initiation of spermatogenesis may therefore be challenged, the requirements underlying the restoration of spermatogenesis in the postpubertal testis is of clinical interest and will be considered here.

A classical approach to examining the gonadotropin requirement of restoration has been to attempt to provide the regressed adult testis with selective FSH or LH stimulation, and to determine whether spermatogenesis is restored with such a monotropic drive. The issue of whether FSH alone may restore spermatogenesis, however, remains unclear. Although treatment with hFSH for 8 wk to GnRH-R antagonist-treated adult macaques stimulated the germinal epithelium with the appearance of premeiotic cell types and a few primary spermatocytes, spermatogenesis was not restored (128). However, it should be noted that, in the latter study, a few cells classified as elongated spermatids were found in 20% of the animals using flow cytometry. In contrast, in normal men rendered hypogonadotropic by im injections of a T ester that produced elevated circulating levels of this steroid (11–14 ng/ml), replacement with human pituitary FSH for 3 months resulted in the reappearance of sperm in semen (133). That spermatogenesis was restored by FSH in men, but not in male monkeys, probably reflects differences in the strategies employed to effect suppression of FSH and LH rather than a fundamental species difference. In the hypogonadotropic men, intratesticular T concentrations during FSH stimulation would have been greater than those in the monkey, and may have therefore contributed to the process of restoration.

The evidence that LH stimulation alone can restore spermatogenesis is compelling. More than 50 yr ago, P. E. Smith (6) reported that implantation of T directly into the testis of hypophysectomized monkeys led to restoration in the vicinity of the implant. In pituitary stalk-sectioned monkeys, T replacement that elevated circulating levels of this steroid to concentrations 10-fold greater than those observed in normal adult monkeys partially restored testicular volume and sperm count to pre-stalk section values (134). In a study with hypophysectomized monkeys, in which intratesticular T content was fully restored, testicular volume recovered to 40% of the presurgical value and 15–20 spermatids per cross section were found (135). The latter parameter may be com-

pared with a value of approximately 40 spermatids per cross section from testis of normal adults in our colony (G. R. Marshall, personal observation). Sertoli cell number was similar in the two conditions. In analogous studies in normal men in which the negative feedback action of exogenous T treatment was employed to induce hypogonadotropism, either hCG or hLH treatment restored spermatogenesis, although sperm production remained below normal (136, 137).

F. Regulation of sperm number

Although spermatogenesis in primates may be maintained by intratesticular T produced in response to LH stimulation of the Leydig cell, it is generally recognized that combined stimulation with FSH and LH, as is the case under physiological circumstances, leads to maximal sperm production (109, 111). After hypophysectomy in the monkey and concomitant initiation of T replacement, testicular size declined to 60% or less of presurgical size, and the number of all germ cells more mature than Ap spermatogonia were reduced (115). Similarly, in normal men, a selective FSH deficiency induced indirectly by the chronic administration of hCG was associated with a marked reduction in sperm count, which was largely restored by treatment with FSH (117). The application of immunoneutralization to selectively decrease the FSH drive to the testis has been used extensively in adult male bonnet monkeys by Moudgal and colleagues (118, 121, 122, 127, 138). Active immunization with ovine FSH, or passive immunization with antiovine FSH, resulted in depletion of the germinal epithelium, a consistent and marked decline in sperm count, and occasional azoospermia in the apparent absence of an effect on LH and T secretion. Analogous studies of the rhesus monkey also indicate a reduction in spermatogenesis after immunoneutralization of circulating FSH (119, 120, 139). Similarly, a reduction in FSH drive to the testis of the adult bonnet monkey, produced by immunoneutralization against the FSH-R in a limited number of animals, also led to a reduction in spermatogenesis (123). In a single study of five human subjects actively immunized with ovine FSH over a 10-wk period, a decline in sperm count was reported to occur during and after the period of immunization (124).

Whereas a selective decrease in FSH drive to the seminiferous tubule leads to a decrease in spermatogenesis, an increase in FSH stimulation appears to amplify the process. Although FSH treatment of oligospermic men has not consistently produced robust increases in sperm count (140–146), certain patients have been reported to respond to FSH stimulation (143–146). Administration of FSH to normal adult monkeys enhanced testicular germ cell number (147), and an enlargement in testicular volume in response to FSH treatment has been reported in men with idiopathic infertility (140). Moreover, a selective elevation in endogenous FSH secretion elicited in the monkey by unilateral orchidectomy is also associated with amplification of spermatogenesis, as reflected by an approximately 70% increase in volume of the remaining testis (148, 149), an increase in the diameter of the seminiferous tubule, and an increase in the number of all germ cells more mature than type Ap spermatogonia (Fig. 4) in the absence of a change in Sertoli cell number (148).

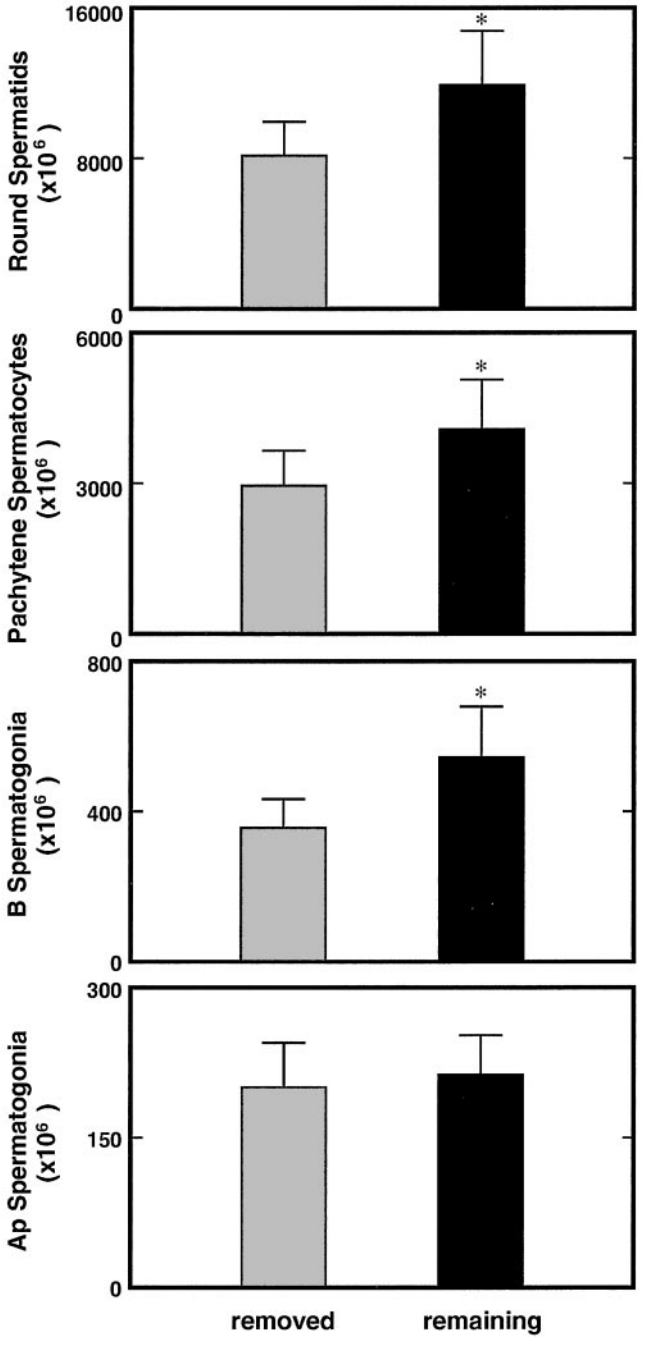

FIG. 4. The mean number of Ap spermatogonia and differentiated spermatogonia (B_1, B_2, B_3, and B_4) per cross section in the testis, which was removed from adult male rhesus monkeys at the time of unilateral orchidectomy (*stippled bars*, removed testis), and in the remaining testis collected 44 d later (*black bars*, remaining testis). The *horizontal bar* indicates the SD, and the *asterisk* indicates a significant difference from the removed testis. [From: S. Ramaswamy *et al.*: *Endocrinology* 141:18–27, 2000 (148). Reprinted with permission of The Endocrine Society.]

Interestingly, as shown in Fig. 5, although total germ cell number was not correlated to Sertoli cell number in the removed testis, in accord with an earlier finding (150), this relationship became highly significant in the remaining testis. Although unilateral orchidectomy is performed in cases

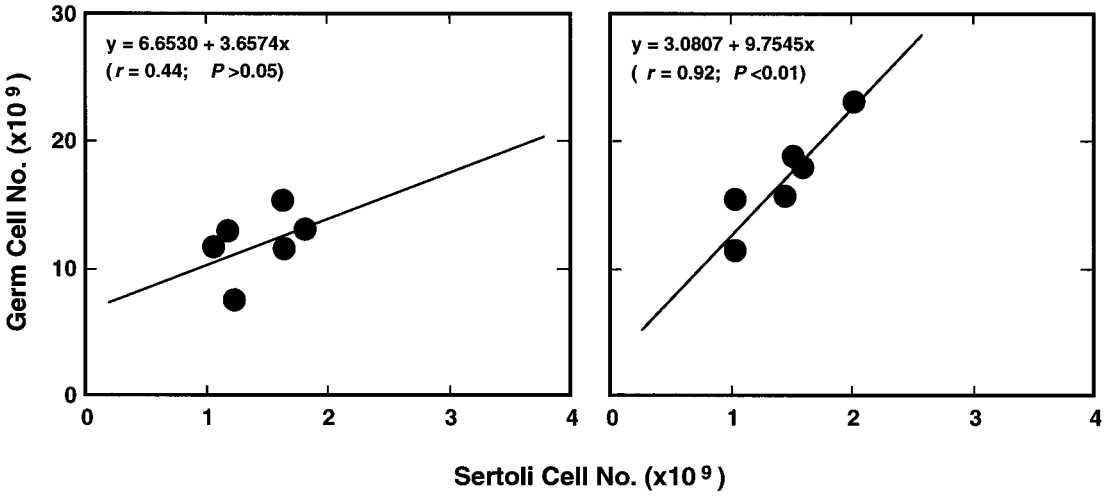

FIG. 5. Relationship between Sertoli cell number and total germ cell number in testes removed from adult rhesus monkeys at the time of unilateral orchidectomy (*left panel*), and when the remaining testes were removed 44 d later (*right panel*). Note the high correlation between the two cell types in the testis remaining after unilateral orchidectomy, which suggests that, at this time, it was operating closer to the spermatogenic ceiling than it was before unilateral orchidectomy. [From: S. Ramaswamy *et al.*: *Endocrinology* 141:18–27, 2000 (148). Reprinted with permission of The Endocrine Society.].

of testicular cancer, torsion, and trauma, it is difficult to generalize on the impact of this surgical procedure on the remaining testis because of the heterogeneity of the subjects and the lack of information on testicular function before unilateral orchidectomy (151–155). In the context of the present review, however, it is interesting to note that sperm counts comparable to those in normal men have been reported in association with elevated FSH concentrations in as many as 20% of patients after removal of a malignant testis (151, 155), and testicular hypertrophy was noted from 6 months to 30 yr (mean, 5 yr) after unilateral orchidectomy in response to accidental injury (152).

Taking the foregoing considerations together, it seems reasonable to propose that the rate of sperm production by the normal primate testis is regulated by the circulating concentration of FSH, and that under physiological situations, blood levels of this gonadotropin are insufficient to drive spermatogenesis to its ceiling.

Conceptually, the action of FSH to amplify a basal level of spermatogenesis driven by intratesticular T may be viewed to proceed according to one of the two models shown in Fig. 6, which are based on the studies by Zirkin and his colleagues (156–158) and indicate that, in the rat, intratesticular T content is maintained at a level in excess of the threshold value required to maintain quantitatively normal spermatogenesis in this species. In the first scenario, it is posited that the primate testis, in contrast to that of the rat, cannot be driven to its spermatogenic ceiling with T alone, and maximal sperm production is achieved only with combined FSH and LH (T) stimulation (Fig. 6, *top panel*). In the second scenario, the relationship between intratesticular T content and sperm output in the primate testis is posited to be similar to that in the model of the rat proposed by Zirkin *et al.* (156). In contrast to the rat, however, the intratesticular content of the adult primate is less than that required to drive the testis to its spermatogenic ceiling, but the latter may be achieved when the seminiferous tubule is provided with a combined LH (T) and FSH stimulation (Fig. 6, *bottom panel*). Here, it is relevant to interject that studies to date have failed to demonstrate that FSH amplifies LH-stimulated T production by the primate testis (159, 160). It will be important to determine which of these paradigms correctly describes the role of FSH in regulating primate spermatogenesis, because resolution of this issue should lead to insight into the molecular mechanisms underlying the hormonal drive to the Sertoli cell. For example, if the first scenario is shown to be applicable, it would be difficult to argue for the view that the FSH-R and AR signaling pathways intersect to regulate transcriptional events common to both pathways (161). At the present time, we favor the first scenario because there is no evidence indicating that intratesticular T alone is able to drive the primate testis to its spermatogenic ceiling, or that FSH is able to enhance the sensitivity of the primate Leydig cell to LH stimulation (159, 160).

The identity of the germ cell(s) that responds to the action of FSH to amplify LH-driven spermatogenesis in the monkey is controversial. In our laboratory, administration of hFSH to T-treated, hypophysectomized adult male rhesus monkeys produced a selective amplification of all four generations of differentiated spermatogonia without a change in the number of undifferentiated Ap spermatogonia (135). This finding led us to propose that the action of FSH is exerted on the first and perhaps subsequent generations of B spermatogonia, a conclusion supported by the more recent observation that the hypersecretion of endogenous FSH elicited by unilateral orchidectomy in this macaque was also associated with an amplification of the four generations of differentiated spermatogonia, again in the absence of a change in undifferentiated Ap spermatogonia (148). In an earlier study of the intact cynomolgus and rhesus monkey, however, FSH stimulation was reported to lead to an increase in both the number of B spermatogonia and Ap spermatogonia, and the authors concluded that the primary effect of FSH was on the undifferentiated spermatogonia (147, 162). As we have dis-

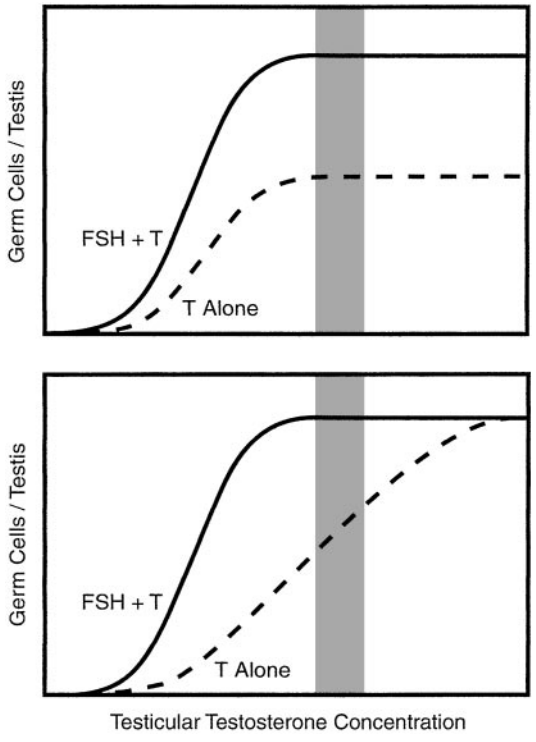

FIG. 6. Two hypothetical models to account for the interaction between FSH and LH (T) to maintain spermatogenesis. The relationship between germ cell number (*abscissa*) and intratesticular content (*ordinate*) is shown when the seminiferous tubule is stimulated by T alone (*broken line*) or by FSH and T in combination (*continuous line*). The *shaded vertical bar* represents the physiological intratesticular T content. In the first paradigm (*top panel*), intratesticular T is maintained at a level that, in the absence of FSH, results in maximal androgen-dependent germ cell production. Here, spermatogenic ceiling may only be achieved when FSH stimulation is combined with that of androgen. In the second model (*bottom panel*), intratesticular T content is maintained at a subthreshold level, and spermatogenic ceiling may be reached by an increase in either the FSH or LH (via intratesticular T) drive to the testis. The conceptual basis for these models was provided by the studies of Zirkin and his colleagues (156–158) describing the relationship between intratesticular T content and germ cell number in the rat.

cussed previously (135), however, the temporal relationship observed by van Alphen *et al.* (147) between the increase in the number of Ap spermatogonia and the increase in B spermatogonia posited to result from the expansion of the population of Ap spermatogonia did not conform to what is known of the kinetics of spermatogenesis in these macaques (53, 56). Specifically, if amplification of B_4 spermatogonia were to result from a prior increase in Ap spermatogonia, then a sustained increase in the number of this stem cell (Ap spermatogonia) would have to be manifest in the preceding cycle of the seminiferous epithelium, which is at least 16 d before the noted amplification of B_4 spermatogonia. Empirically, however, this was not the case, as evaluation of the testis 9 d before the observed amplification of B_4 spermatogonia failed to show an increase in Ap spermatogonia (147).

This Dutch group (147, 162) rationalized their view of the action of FSH on Ap spermatogonia with our finding that FSH stimulation did not influence the number of Ap spermatogonia in the T-treated hypophysectomized monkey (135) by speculating that, in the absence of FSH, the proportion of Ap spermatogonia dividing is reduced. Such a hypothetical action of FSH, however, is unable to account for the FSH-induced amplification of the population of differentiated spermatogonia in the normal testis. This is because it is recognized that, in the macaque and other monkeys, the entire population of Ap spermatogonia divide in stage IX of the cycle of the seminiferous epithelium (53, 55), and therefore, in these species, an increase in the mitotic activity of this cell type is theoretically impossible because it would violate the concept of an invariant cell cycle time. Moreover, in the monkey, the number of Ap spermatogonia does not diminish as these cells age as spermatogenesis progresses from stage IX to stage VIII of the subsequent cycle (53, 55), indicating that survival of this stem cell is robust. If the foregoing two premises are accepted, then in the normal rhesus monkey, an action of FSH on Ap spermatogonia to increase the population of this stem cell is untenable. A similar argument may be made for the cynomolgus monkey, although in this macaque, there are two schools of thought regarding the precise mechanism used for renewal of Ap spermatogonia. According to the view of Clermont and Antar (53), the rhesus and cynomolgus monkeys are identical in this regard, whereas the results of Fouquet and Dadoune (57) suggest that mitosis of Ap spermatogonia occurs at two stages of the seminiferous cycle (stages VII and X). In stage VII, division leads to the generation of daughter Ap spermatogonia, half of which will divide at stage X to produce differentiated B_1 spermatogonia, whereas the other half survive to divide again in stage VII of the subsequent cycle. Although the latter scheme has been accepted by others (150, 163), it should be noted that the number of B_1 spermatogonia observed in stages XI and XII by Fouquet and Dadoune (57) was twice the number of Ap spermatogonia counted in the preceding stage of the cycle (stage X) and, therefore, in 2-fold excess of the theoretical number. Nevertheless, in the cynomolgus monkey there is general agreement that all Ap spermatogonia divide during each cycle (53, 57), and thus, the same theoretical considerations that were argued for the rhesus monkey may also be applied to the cynomolgus macaque.

As a second possibility to account for the observed increase in the number of Ap spermatogonia in response to FSH treatment, van Alphen *et al.* (147) also proposed that a transient transformation of Ad spermatogonia to Ap spermatogonia, followed by restoration in the number of Ad spermatogonia, may be activated by the gonadotropin. Although a transient action on Ad spermatogonia may not be excluded, evidence for such an effect of FSH stimulation is not at hand, and a transient restoration of the population of Ad spermatogonia is difficult to conceptualize. Therefore, after re-examining the forgoing considerations, we remain of the opinion that the most parsimonious explanation for the physiological relationship between FSH drive on the one hand and the rate of testicular sperm production by the primate testis on the other is that the gonadotropin exerts an action on the first, and perhaps subsequent, generations of differentiated spermatogonia to amplify the population of these cell types. In consequence, the number of all more mature germ cells is enhanced.

Before discussing the mechanism whereby FSH amplifies the population of B spermatogonia, it is necessary to reiterate that the sequential steps in the production of testicular spermatozoa from undifferentiated spermatogonia is recognized to unfold according to a fixed kinetic program, with the cell cycle of each type of dividing germ cell and the morphogenic transformation during spermiogenesis being immutable (52, 67). It follows from this dogma, therefore, that the action of FSH to amplify the population of B spermatogonia must be attributed to an ability of the gonadotropin to promote survival of at least the first generation of these differentiated germ cell types. The most likely mechanism whereby this may occur is that the FSH directly governs the secretion of a Sertoli cell factor that suppresses apoptosis of this differentiated cell type. The nature of this paracrine control by the Sertoli cell of differentiated spermatogonia in primates has yet to be addressed, but it may be anticipated to use the factors that have been implicated to be operative in cognate systems regulating spermatogonial survival in the testis of nonprimate species (164–173).

Returning now to the role of gonadotropin in maintaining the population of Ap spermatogonia in the adult testis, the following may be noted. In adult male cynomolgus monkeys rendered hypogonadotropic for several weeks by hypophysectomy or treatment with either a GnRH-R antagonist or T, a marked reduction in the total number of Ap spermatogonia was generally observed in association with a profound depletion in differentiated germ cells (115, 128, 163, 174). Additionally, the gonadotropin dependency of the pubertal proliferation of Ap spermatogonia in the monkey has been noted earlier (31). On the other hand, a decrease in the number of Ap spermatogonia was not observed in five normal men after suppression of gonadotropin secretion during 19–24 wk of treatment with T enanthate, although the hypogonadotropic state produced a marked reduction of all germ cells more mature than undifferentiated spermatogonia (175).

That a loss of FSH action may have contributed to the reduction of Ap spermatogonia observed by Weinbauer *et al.* (128) in response to chemical hypophysectomy in the cynomolgus monkey was indicated by the finding that initiation of an 8-wk period of FSH treatment at the start of GnRH-R antagonist administration prevented the depletion of Ap spermatogonia. Similarly, it would appear that LH action may also maintain the population of Ap spermatogonia because, in the cynomolgus monkey, the number of this cell type in the testis of T-treated, hypophysectomized animals is similar to that in the intact situation (115). In summary, extant data would suggest that an action of either FSH and/or LH is required to maintain the population of Ap spermatogonia of the normal adult testis. At the time of writing, we view this action of gonadotropin on Ap spermatogonia to be permissive, allowing Ap spermatogonia to divide each cycle and to survive. Such an action of gonadotropin is to be contrasted with the regulatory action of FSH that dictates the number of differentiated spermatogonia that survive. Clearly, further study of the role of the gonadotropins in this regard is needed.

G. Determination of sperm quality

In the final analysis, the quality of sperm is best confirmed by successful fertilization and implantation, events that are not easily amenable to quantitation in primates. Nonetheless, a few studies merit discussion. Most notably, active immunization of bonnet monkeys against ovine FSH resulted in an impairment of sperm motility and in a decrease in the activity of acrosin, an acrosomal enzyme that plays a role in the penetration of the oocyte, and the immunized monkeys failed to impregnate fertile females in a well controlled mating test (138). Moreover, immunoneutralization of circulating FSH in man and monkey is associated with an alteration in the integrity of sperm chromatin and a reduction in the glycoprotein content of the acrosome (176, 177), which in the case of the latter, may underlie the decrease in acrosin activity reported earlier (138). Furthermore, several fertility centers have reported that FSH treatment of infertile, oligospermic, or teratozoospermic men for 1–3 months results in an increase in the number of sperm with normal ultrastructural characteristics (141, 142, 144), and in one report, FSH treatment was associated with an increase in fertilizing efficiency of sperm *in vitro* (178). Clearly, there is a need for an experimental model in which a selective and absolute FSH deficiency may be induced postpubertally to examine the role of FSH in determining sperm quality.

V. Regulation of FSH Secretion

In adult men and male macaques, the central neural drive for FSH secretion, like that for LH synthesis and release, is provided by a network of GnRH-expressing neurons in the hypothalamus (179). This network of GnRH neurons with its attendant afferent neuronal and glial inputs (180) is referred to as the GnRH pulse generator (181, 182). This neuroendocrine system generates an intermittent discharge of GnRH into the hypophysial portal circulation (183), and this episodic stimulation of the pituitary gonadotrophs is obligatory for sustained FSH and LH secretion (184). When this hypophysiotropic drive is congenitally absent, as in the case of Kallman's syndrome, or is abolished experimentally in the adult male monkey, circulating FSH concentrations are very low and often undetectable (185, 186). Although LH secretion in men and male macaques is sensitive to changes in the frequency of pulsatile GnRH stimulation (185, 187), which under physiological conditions, is dictated by a feedback action of testicular T secretion to retard the hypothalamic GnRH pulse generator (179), the release of FSH, as reflected by mean concentrations of the hormone in blood, appears to be relatively unresponsive to GnRH frequency modulation. In studies by Crowley and his colleagues (188) of men in whom hypothalamic GnRH release was severely compromised, pulsatile stimulation with exogenous GnRH replacement at frequencies ranging from 1 to 8 pulses every 2 h was not associated with changes in plasma FSH concentrations (Fig. 7). Additional slowing of the frequency of GnRH stimulation in similar patients to 1 pulse every 8 h also failed to enhance FSH secretion (189). In one other study of men with idiopathic hypogonadotropic hypogonadism, however, circulating FSH levels during GnRH stimulation at 1 pulse

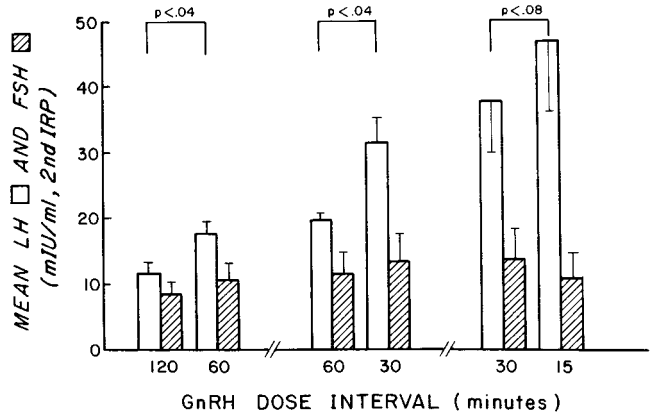

FIG. 7. Circulating FSH concentrations (mean ± SEM, *cross-hatched bars*) in five GnRH-deficient men failed to respond to successive stepwise increments in frequency of exogenous GnRH stimulation provided by an intermittent iv infusion of an invariant dose of synthetic peptide (17–22 ng/kg body wt) administered at interpulse intervals of 15–120 min. The corresponding LH levels are shown by the open bars. [From: D. I. Spratt *et al.*: *J Clin Endocrinol Metab* 64:1179–1186, 1987 (188). Reprinted with permission of The Endocrine Society.]

every 90 min was greater than that at the faster frequency (190). The reasons for the different FSH response reported in these two groups of men with idiopathic hypogonadotropic hypogonadism is unclear, although it has been proposed that the extent to which T levels were normalized may be a contributing factor (190). In the hypothalamic-lesioned adult male rhesus monkey, which may be viewed as analogous to the human paradigm, an increase in GnRH pulse frequency 3 wk after castration from 1 pulse every 3 h to 3 pulses every 3 h did not stimulate FSH secretion, but did lead to a robust elevation in LH concentrations (185). Similar results were also obtained in the juvenile cynomolgus monkey when GnRH frequency changes were imposed in the presence of T (191). Taken together, the foregoing studies lead us to propose that FSH secretion in the male primate is relatively emancipated from modulation by the frequency of the hypophysiotropic drive. This may be in contrast to the situation in the male rat, in which FSHβ gene expression is markedly modulated by changes in GnRH frequency (192).

On the other hand, direct testicular negative feedback on FSH secretion at the level of the gonadotroph is profound in the adult male monkey, as reflected by the FSH response to bilateral orchidectomy in an experimental paradigm known as the hypophysiotropic clamp (193). In this preparation, the endogenous hypophysiotropic drive to the gonadotroph is interrupted either experimentally or by activation of a normal physiological control system. In the former case, the hypothalamus of the adult is lesioned to abolish pulsatile GnRH release (185). In the latter, a juvenile monkey in which pulsatile GnRH release is held in check by a developmental brake, is used (194). In both types of animals, an adult-like pattern of hormonal activity may be elicited by an intermittent iv infusion of GnRH. Because the hypophysiotropic drive to the gonadotroph is clamped in these preparations, any change in gonadotropin secretion resulting from a perturbation to testicular feedback signals must be accounted for by a change in feedback directly at the level of the pituitary. Bilateral orchidectomy in this model results in a selective and dramatic hypersecretion of FSH (Fig. 8), which is not prevented by T replacement at the time of castration (194, 195). *In vitro* studies employing primary pituitary cell cultures have also failed to demonstrate an action of T on FSH secretion directly at the level of the monkey gonadotroph (196). Moreover, because passive immunoneutralization against circulating E2 in the testicular intact hypophysiotropic clamp fails to elicit an increase in FSH secretion (195), it may be concluded that the specific testicular FSH-inhibiting factor is nonsteroidal. That this factor is inhibin was suggested by the finding that passive immunoneutralization of circulating inhibin in the testicular intact clamp results in an elevation in FSH secretion similar to that observed after castration (197). This notion was greatly reinforced by the finding that, in this experimental paradigm, initiation of a continuous iv infusion of rh inhibin A at the time of bilateral orchidectomy prevented the postcastration hypersecretion of FSH and the increase in levels of mRNA encoding the FSH β-subunit (198). A direct effect of inhibin at the monkey gonadotroph to suppress FSH secretion has also been demonstrated *in vitro* (196).

Thus, the foregoing studies of the hypophysiotropic clamp have led to the conclusion that, in the monkey, the testicular regulation of FSH secretion is governed by a control system consistent with that described by the classic inhibin hypothesis (199). This view was fully confirmed by studies of the normal intact adult male monkey. Most notably, passive immunoneutralization of circulating inhibin in the intact adult male results, as in the clamp, in a hypersecretion of FSH (200), and a continuous iv infusion of rh inhibin A results within 54 h in a significant suppression of circulating FSH

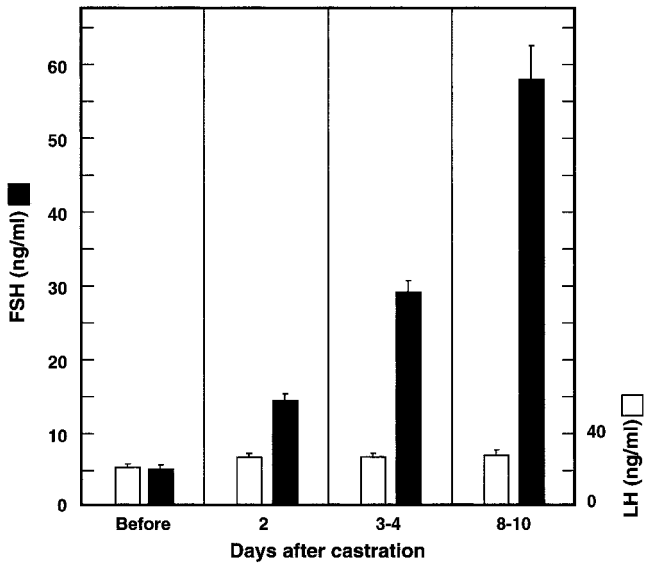

FIG. 8. The effect of bilateral castration on d 0 on gonadotropin secretion in adult male rhesus monkeys bearing hypothalamic lesions, in which activity in the pituitary-testicular axis was restored with a continuous intermittent iv infusion of GnRH (0.1 μg/min for 3 min every 3 h). Note the dramatic and selective postcastration rise in circulating FSH concentrations (mean ± SE, *filled bars*) in this experimental paradigm. The corresponding LH levels are shown by the *open bars*. [From: T. M. Plant and A. K. Dubey : *Endocrinology* 115: 2145–2153, 1984 (185). Reprinted with permission of The Endocrine Society.]

concentrations without influencing LH secretion (Ref. 201 and Fig. 9). The findings that inhibin B is the principal form of the dimeric hormone in the circulation of the adult male monkey, and is of testicular origin, establishes that inhibin B is indeed the native testicular inhibin in this species (202).

Convincing evidence supporting the premise that FSH secretion in men is regulated by the negative feedback action of testicular inhibin is at last accumulating. Many years ago, it was demonstrated that selective hypersecretion of FSH could exist in the face of normal T levels in men with azoospermia (203). Initial measurement of circulating inhibin levels by RIA in such men failed to reveal the expected inverse relationship between FSH and the testicular protein (204). The inhibin hypothesis suffered a further set back when specific ELISAs for inhibin A, one of two dimeric forms of the mature hormone, failed to detect this molecule in the circulation of normal men (205). It is now known, however, that the original RIA used for infertile men recognizes circulating inhibin α-subunit as well as dimeric inhibin (206, 207), and that dimeric inhibin in the circulation of the human male is accounted for entirely by inhibin B (208–210). Moreover, inhibin B in men is present in substantial concentrations (300–500 pg/ml), is of testicular origin, and is inversely related to circulating FSH levels (209, 210). Thus, it is reasonable to reaffirm the proposal that testicular inhibin is the major gonadal signal regulating the secretion of FSH in the human male, a conclusion that is strongly reinforced by our studies of the rhesus monkey.

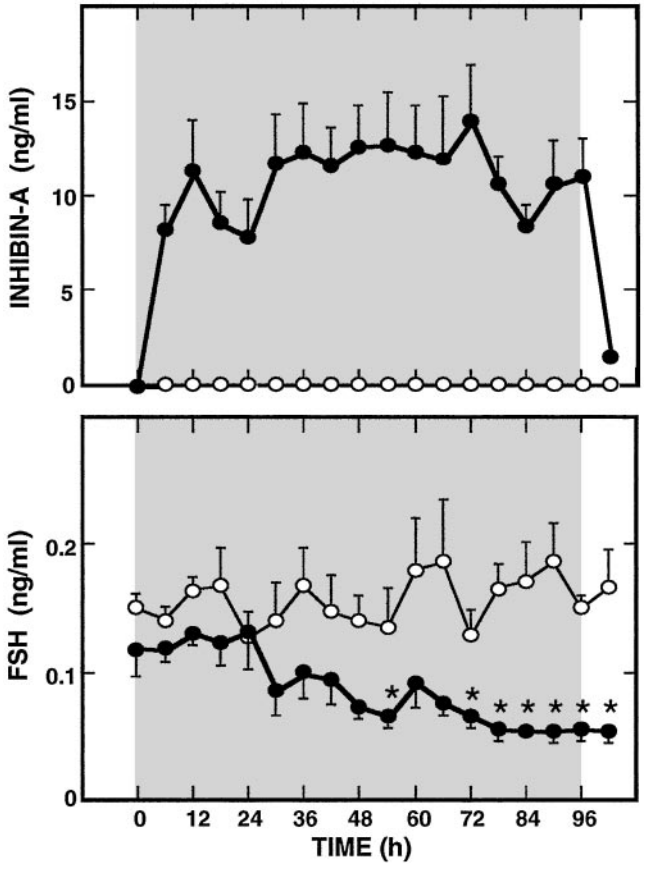

FIG. 9. Infusion of rh inhibin A (0.8 μg/kg·h, *closed data points*) to adult male rhesus monkeys from 0–96 h (*stippled area*), which produced a 10-ng/ml increment in circulating inhibin A concentrations (*top panel*), resulted in a progressive suppression in plasma FSH levels (*bottom panel*). The *open data points* show data obtained from the same animals during infusion of vehicle. Mean ± SE values are shown, and *asterisks* indicate FSH concentrations significantly different from the preinhibin infusion value. [From: S. Ramaswamy *et al.*: *Endocrinology* 139:3409–3415, 1998 (201). Redrawn with permission of The Endocrine Society.].

The action of inhibin B at the level of the primate gonadotroph has not been studied either *in vivo* or *in vitro,* but experiments with rh inhibin A suggest that, in the rhesus monkey, testicular inhibin selectively regulates FSHβ gene expression and FSH secretion (198, 201). To date, the most parsimonious mechanism that may be proposed to account for this action of inhibin B is that this testicular peptide antagonizes a constitutively expressed activin drive to the FSH-secreting gonadotroph, a view consistent with the finding that castration of the adult male monkey does not markedly influence the pituitary level of the mRNA encoding either activin/inhibin βB or follistatin (211, 212). Inhibin B may antagonize the paracrine action of activin by either binding to and inactivating the ligand binding subunit of the activin receptor (213, 214) or by binding to a specific inhibin receptor, which in turn antagonizes the action of activin. With regard to the latter possibility, it may be noted that 1) inhibin binding sites with high affinity and specificity have been described in ovine pituitary cells (215); 2) the type-III TGF-β receptor, betaglycan, has been demonstrated to mediate inhibin antagonism of activin signaling (216); and 3) a specific inhibin binding protein (InhBP) has been cloned from bovine pituitary cells (217). Neither betaglycan nor InhBP, however, has an intrinsic kinase domain (216, 218), and presumably, if these proteins serve as inhibin receptors they must do so by antagonizing the paracrine action of activin. Recent studies by Woodruff and her colleagues (218) using suppression of activin-stimulated gene expression in a transfected cell line as an index of inhibin activity indicate that inhibin B may be the ligand favored by InhBP. If this is the case, it may be anticipated the InhBP will be found to be highly expressed in the gonadotroph of the male primate, a species in which inhibin B is the major testicular regulator of FSH secretion in the adult.

The physiological significance of the FSH-inhibin B feedback loop in the regulation of spermatogenesis in the adult monkey may be demonstrated by removing one testis and examining the impact of this perturbation on the dynamic relationship between the endocrine changes effected on the one hand and the germinal response of the remaining testis on the other (148). As expected, unilateral orchidectomy results in a rapid and permanent deficit in circulating inhibin B levels, which is followed by a robust and sustained increase in FSH secretion in the face of only a very transient perturbation of LH and T secretion (Fig. 10). The hypersecretion of FSH, in turn, is followed by a progressive enlargement of the remaining testis as it is driven toward its spermatogenic ceiling by the increased FSH drive (Fig. 10). It is important to note that, in the monkey, the response of the FSH-secreting gonadotroph to changes in circulating inhibin tone is robust and contrasts with the less sensitive relationship between changes in FSH levels and testicular inhibin B production

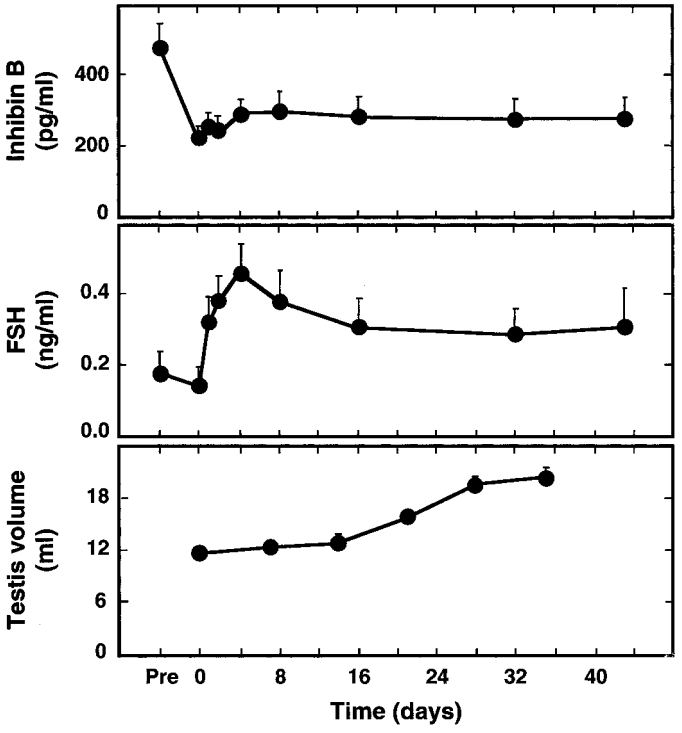

FIG. 10. Changes in circulating inhibin B concentrations (*top panel*), circulating FSH concentrations (*middle panel*), and testicular volume of the remaining testis (*bottom panel*) after unilateral orchidectomy on d 0 in a group of adult male rhesus monkeys. Mean ± SE values are shown. The restoration in circulating inhibin B levels from the immediate postunilateral orchidectomy time point to d 4 was small (~24%) but statistically significant (see Ref. 148). [From: S. Ramaswamy *et al.*: *Endocrinology* 141:18–27, 2000 (148). Redrawn with permission of The Endocrine Society.].

(218). This differential gain in the feedforward (FSH-inhibin B) and feedback (inhibin B-FSH) arms of this feedback loop in the monkey is responsible, after unilateral orchidectomy, for the persistent error signal (a decrease in circulating inhibin B) at the hypophysial level for the release of FSH; it is therefore the key element underlying the ability of this feedback control system to set the level of circulating FSH, thereby regulating the rate of sperm production in the monkey (219). That the FSH-inhibin B feedback loop in men may operate in a manner similar to that described for the monkey is suggested by the finding that whereas chronic FSH treatment of men with idiopathic fertility was associated with an increase in testicular volume, circulating inhibin B levels in these subjects did not respond to FSH stimulation (140). Moreover, in normal men, administration of a large dose of rhFSH that resulted in an approximately 7-fold increase in circulating concentration of the gonadotropin elicited only a fold increase in plasma inhibin B levels (208). Although unilateral orchidectomy in men (see above) is usually associated with elevated FSH secretion, in contrast to the monkey, the hypersecretion of gonadotropin was not restricted to FSH (151, 153–155).

Before closing our discussion on the regulation of FSH secretion, it is important to state that the release of this gonadotropin in male primates is not insensitive to inhibition by testicular steroids. In fact, supraphysiological plasma levels of either T or E2, achieved by administration of exogenous steroid, dramatically suppress circulating FSH concentrations in normal men and male macaques (220–223), probably by exerting actions at both the pituitary and hypothalamic levels (221–225). The relative contribution of such steroid inhibition in the feedback control system governing FSH secretion in a physiological setting, however, appears to be noticeably less important than that produced by the inhibin B signal. In normal men, abolishing testicular steroidogenesis with ketoconazole, although largely preserving the inhibin B tone in these subjects, resulted in a minor increase (doubling) in circulating FSH concentrations (226). This is to be compared with the dramatically elevated levels of circulating FSH observed in the absence of all testicular feedback signals in castrate or hypogonadal men (227). The steroid component of the testicular inhibition of FSH secretion in men appears to be mediated by ER activation in response to an E2 signal generated either by secretion of the steroid directly from the testis or by peripheral or central neural aromatization of secreted T. This view is based on several findings. First, circulating FSH concentrations in men with mutations of ER-α or aromatase deficiency are greater than those in control subjects (228), and treatment of normal men with anastrazole, a specific inhibitor of aromatase, results in an FSH hypersecretion comparable to that after ablation of all testicular steroid secretion with ketoconazole (226).

VI. Summary

A simple model that describes the role and operation of the FSH-inhibin B feedback loop in the maintenance of spermatogenesis in the testis of the adult primate is shown in Fig. 11. According to this model, the circulating concentration of FSH is posited to provide the signal that sets the level of sperm production above the basal rate induced by intratesticular T. The action of FSH on the germ cells is indirect and mediated by a paracrine signal(s) of Sertoli cell origin that acts as a survival factor for differentiated spermatogonia and therefore amplifies a basal level of spermatogenesis that is maintained by T. FSH secretion, although absolutely dependent on pulsatile GnRH stimulation, is relatively insensitive to frequency modulation of the hypophysiotropic signal, and the rate of FSH secretion is selectively dictated by the negative feedback action of testicular inhibin B secreted by the Sertoli cell. Inhibin B probably suppresses FSH secretion by antagonizing a constitutively expressed activin drive to FSH β gene expression. Although proteins that specifically bind inhibin have been described, the relative importance of these putative receptors or coreceptors in mediating the action of inhibin B at the level of the primate gonadotroph remains to be established. An essential feature of this feedback control system is that the feedback arm of the loop (inhibin B-FSH) is more robust than the feedforward arm (FSH-inhibin B), and thus, a change in the testicular feedback signal (inhibin B) results in a sustained perturbation to FSH secretion. The physiological set point of the feedback loop is such that the circulating concentration of FSH is insufficient to drive the seminiferous tubule to its ceiling of operation. The latter finding is consistent with the notion that the spermatogenic

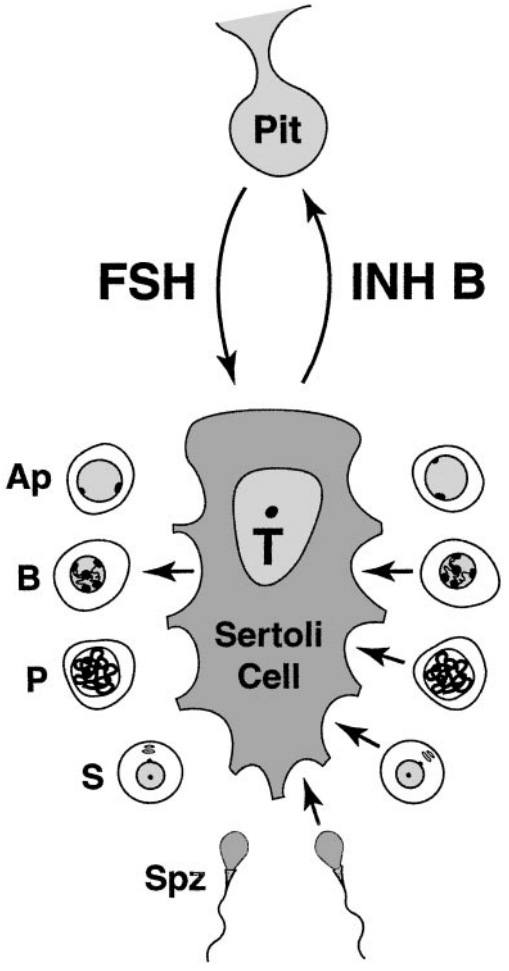

FIG. 11. A model of the negative feedback control system that regulates sperm production by the primate testis. According to this model, FSH amplifies a basal level of spermatogenesis that is dependent on intratesticular T. The degree of amplification is directly related to the circulating concentration of FSH, and the FSH drive is relayed to the germinal epithelium via the production of a paracrine factor by the Sertoli cell. This paracrine factor favors the survival of differentiated B spermatogonia (B), which leads to an increase in the number of subsequent generations of germ cells. The FSH concentration is regulated by the rate of secretion of inhibin B (INH B) by the Sertoli cell. Inhibin B exerts a brake on FSH secretion by suppressing FSHβ gene expression. The mechanism that controls the rate of inhibin B secretion by the testis is controversial but in the present model a signal(s) from the differentiated germ cells is proposed to positively regulate inhibin B production by the Sertoli cell. The intensity of the putative germ cell signal is posited to be related to the number of differentiated germ cells. P, Primary spermatocyte; S, round spermatid; Spz; elongating spermatid and testicular spermatozoa; pit, pituitary gland.

ceiling of the adult primate testis may be set by the size of the population of renewing stem Ap spermatogonia extant upon completion of puberty rather than by the number of Sertoli cells established at this stage of development. Although the pubertal proliferation of Sertoli cells appears to be produced by an combined action of FSH and LH, the endocrine drive underlying division of Ap spermatogonia at this stage of development is less clear. From a physiological perspective, an unresolved question concerns the cell biology that inversely couples production of mature germ cells with inhibin B secretion, but presumably, feedback signals from the germ cells to the Sertoli cell must be involved.

With regard to the issue of fertility control on the one hand and the treatment of infertility in men on the other, we are of the following opinion. From the extant data, it seems reasonable to conclude that interruption of the feedforward arm of this control system in men and other male primates (*i.e.*, abolition of FSH secretion or action) will not prevent either the initiation or maintenance (qualitative) of spermatogenesis and therefore will not lead to azoospermia. The question of the quality of the sperm produced in the absence of FSH, however, remains to be answered. The view that FSH is not required for fertility is epitomized by the contemporary finding that men with an inactivating mutation of the FSH-R can be fertile. Because biological systems exhibit redundancy and plasticity, the impact of the loss of a gene from conception may be later compensated for by an alternate pathway. Indeed, in the monkey, the postpubertal interruption of FSH action using immunoneutralization was found to be associated with infertility. Therefore, in our view, the question of whether a complete and specific abolition of FSH signal transduction imposed after puberty in the human male would be associated with infertility remains to be answered. The issue of a super inhibin receptor agonist as a male contraceptive must await resolution of the latter question. As for male infertility, the value of FSH treatment, particularly in subsets of subjects with idiopathic infertility, should not be dismissed at the present time.

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2002 Prolactin Gordon Research Conference

The next Gordon Research Conference on Prolactin will be held in Ventura, California, from Jan. 27–Feb. 1, 2002. As has been the case for the past several years, we will also address similar issues for growth hormone. There are two highlighted presentations: The meeting begins with a debate on Sunday evening entitled, "Do prolactin and growth hormone cause cancer?" M. G. Rosenfeld will end the meeting with the Keynote Address on Thursday evening, speaking on "Genetic control of pituitary development." Morning and evening sessions during the week will cover various aspects of PRL and GH secretion, receptors, and actions, including mouse models, microarray analysis and proteomics, PRL and GH physiology, pituitary development and regulation, agonists, antagonists and assays, effects in peripheral tissues, behavior and lifestyles, and PRL and GH signaling. There will also be eight Platform Presentations by graduate students, postdoctoral fellows, or junior faculty, who will present late-breaking, up-to-the-minute results from their submitted abstracts. The remaining abstracts will be presented in two poster sessions. For more information on the full program or GRC registration, check the Web site at *http://www.grc.org*.

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