

## Commentary

# The endocrine regulation of spermatogenesis: independent roles for testosterone and FSH

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### Introduction

The adult testis has two important roles, namely the production of spermatozoa (fertility) and the secretion of testosterone which is needed for the expression of secondary sexual characteristics (virility). These functions depend on stimulation by the pituitary gonadotrophins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which are stimulated by hypothalamic gonadotrophin-releasing hormone (GnRH). Testosterone is secreted by the Leydig cells under LH stimulation and is essential for promoting spermatogenesis. While FSH has a role in the development of the immature testis (Orth 1993), controversy persists as to whether FSH is essential for the maintenance of adult spermatogenesis. A better understanding of the hormonal requirements of adult spermatogenesis is needed for the development of rational treatments for human infertility and in designing contraceptive strategies.

This review will outline the data regarding the roles of testosterone and FSH in adult spermatogenesis and follows previous discussion in this Journal (Rommerts 1988, Sharpe 1989). While the initiation of spermatogenesis during puberty is not considered, it will be argued that both testosterone and FSH each have independent actions in the maintenance of normal spermatogenesis in adulthood.

### Gonadotrophin dependency of spermatogenesis

When gonadotrophins are withdrawn from the adult animal sperm production is abolished. This outcome can be achieved by hypophysectomy (Clermont & Harvey 1965), GnRH immunization (Awoniyi *et al.* 1989), GnRH analogue treatment (Rea *et al.* 1986) and the suppression of gonadotrophins by exogenous androgens (Robaire *et al.* 1979). The controversy regarding the need for FSH in adulthood stems primarily from the observation that adult spermatogenesis can be restored by the replacement of testosterone alone in rats made gonadotrophin-deficient by immunization against GnRH (Awoniyi *et al.*

1989) and by the reported failure of spermatogenesis to be disrupted by short-term passive immunization against FSH (Raj & Dym 1976). Yet in primates (including man) an essential role for FSH in the maintenance of spermatogenesis has been shown (Srinath *et al.* 1983, Matsumoto *et al.* 1986).

A multitude of factors probably accounts for these different conclusions. A key issue is that spermatogenesis is difficult to study *in vitro* due to the inherent complexity of germ cell development and the interaction between the germ and somatic cells. Complete germ cell maturation has not been achieved *in vitro* and thus these studies have limited relevance to events *in vivo*. The recent reports of spermatogonial and spermatogenic cell lines which undergo meiosis *in vitro* promise to be exciting developments (Hofmann *et al.* 1994). Inconsistencies in the *in vivo* data may be partly due to the wide range of experimental designs and the use of different species. The major technical issue is how best to describe hormonally induced changes in spermatogenesis. Only recently have contemporary stereological techniques of cell quantification been used as the endpoint in studies on the spermatogenic process.

In order to clarify these issues it is necessary to use well-characterized animal models in combination with germ cell counting techniques which allow quantitation of the effects of hormones on the germ cell lineage and the consequent identification of the cellular sites of testosterone and FSH action. Such a paradigm provides the essential first step in understanding the mechanism by which testosterone and FSH regulate spermatogenesis. Examples of these models and cell quantification procedures will be described prior to the discussion of testosterone and FSH action *in vivo*.

### *In vivo models for the study of spermatogenesis*

All *in vivo* models have limitations which need to be appreciated when interpreting data. The hypophysectomized rat provides a model of complete gonadotrophin

withdrawal; however, the data are clouded by the concomitant loss of other pituitary hormones. Consequently the use of models in which gonadotrophin release is specifically modulated are generally preferable. The two most widely employed models are as follows.

(1) Testosterone-suppressed rat model. Exogenous testosterone is given by Silastic implant to obtain slightly supraphysiological serum testosterone levels and results in the suppression of LH and intratesticular testosterone levels. It should be noted that testicular testosterone levels are normally ~40-fold higher than serum levels due to its local production. Following LH withdrawal, testicular testosterone levels fall to those in normal serum, i.e. ~3% of normal (Zirkin *et al.* 1989). Low doses of oestradiol can be given in combination with testosterone as it has a synergistic effect in suppressing testicular function, presumably due to the more profound suppression of LH levels (Robaire *et al.* 1979). Serum FSH levels show little change in response to testosterone plus oestradiol implants, indicating that this model is primarily one of LH/testicular testosterone deficiency (McLachlan *et al.* 1994a). It is interesting that the suppression of FSH levels in adult rats has been reported when testosterone was given by injection (Sharpe *et al.* 1988); however, the basis for this different response is unclear. When high doses of testosterone implants are then given (achieving serum levels five times normal), spermatogenesis can be restored as testicular testosterone levels rise to ~20% of normal (Zirkin *et al.* 1989). The study of germ cell numbers in this model allows for the identification of the critical sites of testosterone action in the presence of FSH (see below).

(2) GnRH withdrawal in adult rats. There are two approaches to withdrawing GnRH action *in vivo*. Active immunization against GnRH renders serum gonadotrophin levels undetectable and reduces serum testosterone into the castrate range (Awoniyi *et al.* 1989, McLachlan *et al.* 1994b). GnRH analogue treatment has similar effects (Sinha-Hikim & Swerdloff 1993), although this approach is more laborious and expensive, especially in long-term experiments. Irrespective of the method, it should be noted that, even though serum LH levels are undetectable using a sensitive immunofluorometric assay (Haavisto *et al.* 1993), the possibility of residual FSH activity is difficult to exclude with the current insensitive FSH RIAs.

Spermatogenesis is grossly impaired in the GnRH-immunized (McLachlan *et al.* 1994b) or GnRH antagonist-treated rat (Sinha-Hikim & Swerdloff 1993), with few cells progressing beyond the primary spermatocyte stage. Nonetheless, the spermatogenic process can be promptly restored by testosterone even after many weeks, indicating that a stable, albeit severely reduced, spermatogonial population is maintained (McLachlan *et al.* 1994b). While this model seems ideal for the independent study of testosterone and FSH on the restoration of

spermatogenesis, it does suffer from one problem peculiar to rodents, i.e. the effect of testosterone treatment to restore serum FSH levels by GnRH-independent effects on pituitary FSH secretion (Rea *et al.* 1986, Bhasin *et al.* 1987), although this effect has not always been observed (Awoniyi *et al.* 1989). Consequently the effects of testosterone on the restoration of spermatogenesis cannot be studied free from those of FSH. Conversely, however, the effects of FSH on the restoration of spermatogenesis can be studied independently of those of testosterone (see below).

#### *Quantitation of spermatogenic cells in vivo: the stereological approach*

When evaluating hormonal effects on spermatogenesis, there is a critical need for quantitative rather than qualitative or relative data on germ cell numbers. The cell types involved in spermatogenesis are amenable to objective and reproducible quantitation on a per testis basis, yet few studies have applied sufficiently rigorous modern techniques for determining cell number. These procedures form part of the discipline of stereology and their application to the testis has been reviewed by Wreford (1995). Stereological techniques allow the spermatogenic lesion associated with a particular hormonal manipulation to be localized to particular cell types or groupings. Some authors advocate quantifying germ cell changes by counting nuclear profiles per tubule cross-section only at defined stages (most often stage VII due to its well-known hormone dependency); however, this may overlook significant changes that occur at other stages. Other authors report counts as a ratio of germ cell to Sertoli cells. Both techniques result in a ratio in which the denominator is assumed to be constant. We prefer to assess the total testicular germ cell content so that the whole process of spermatogenesis can be considered. Two stereological approaches to germ cell counting are briefly outlined, each involves the quantification of cell nuclei and assumes that this equates directly to cell number.

**Geometric model** This is the traditional approach based on the Floderus equation and is predicated on the assumption that the nuclei are spherical and homogeneous in size (reviewed in Wreford 1995). This assumption is reasonable in the case of pachytene spermatocytes and round spermatids but not of elongated spermatids. Using 2  $\mu\text{m}$  sections, estimates of profile areal density ( $N_A$ ) are made, then extrapolated to numerical density ( $N_V$ ) using diameter, section thickness and 'lost cap' data; the latter are glancing sections of nuclei which are not visible in the section. Estimates of the  $N_V$  of cell nuclei can be used in conjunction with estimates of the reference volume of the processed tissue to determine the total number of germ cells in a particular category.

**Optical disector method** This recent stereological innovation was described by Gundersen *et al.* (1988) and utilized by Braengaard *et al.* (1990) to estimate neuronal number. The technique involves the direct counting of nuclei in defined volumes contained within thick (25  $\mu\text{m}$ ) methacrylate sections. Nuclei are counted as their equatorial regions, or some other unique features, come into focus. The most significant advantage of this method is that it is not affected by either particle size or shape. It thus permits the estimation of non-spherical objects such as elongated spermatid nuclei (by counting their tips). The  $N_v$  for each cell type is calculated by dividing the number of cells counted by the volume of the disector (the area of the counting frame multiplied by the depth scanned). The resulting estimates of  $N_v$  can then be extrapolated to estimate total cell number as described above.

**Sampling schemes** The systematic uniform random sampling technique, described by Gundersen & Jensen (1987), facilitates the determination of average numerical density independent of the considerable stage heterogeneity observed in the testis. The basic principle of the scheme involves sampling fields at uniform intervals from a random starting point. This sampling scheme ensures that cells are counted efficiently with a probability proportional to their prevalence. Accordingly, germ cells present in stages of longer duration are sampled more frequently. This approach is widely applied in stereological studies in heterogeneous tissues of similar complexity to the testis, such as the central nervous system (Braengaard *et al.* 1990).

Using the above methods, the total number of germ cells can be calculated independent of changes in tubule dimensions, Sertoli cell nuclear diameter and Sertoli cell number. One limitation is that the staging of spermatogenesis becomes difficult in the severely regressed testis, making accurate cell identification difficult, e.g. the differentiation of type B spermatogonia from preleptotene spermatocytes. This limits the number of separate cell categories and therefore the precision with which the site of hormone action can be defined.

Some investigators express data in terms of germ cell/Sertoli cell ratios. Such ratios are critically dependent upon two propositions: (i) that Sertoli cell number is invariable, a concept supported by the majority of studies using a range of hormonal manipulations, and (ii) that the size and shape of the Sertoli cell nuclei or nucleoli used in the estimation of cell number is also unaffected. The latter assumption may well be flawed as, for example, Sertoli cell nuclear volume decreases to 30% of control values following GnRH immunization (McLachlan *et al.* 1995), corresponding to a decrease in mean diameter to 67% of control. This reduction in diameter leads to a reduced probability of Sertoli cell nuclei being sampled in the sections relative to the germ cells, which do not show a

similar reduction in nuclear size. Making some assumptions, e.g. that shrinkage of Sertoli cell nuclei is uniform, errors in the order of 30% in the germ cell/Sertoli cell ratios may result from such unrecognized changes. This problem has been recognized and may be partially avoided by correcting for Sertoli cell size or using nucleoli which are claimed to be less sensitive to hormonal changes.

#### *Spermatogenic cycle kinetics*

The absolute number of germ cells per testis can be used to follow the progression of spermatogenesis using calculated hourly production rates (HPR). This involves the utilization of time divisors which take into account the proportion of the spermatogenic cycle (12.9 days) represented by the stages at which the germ cells are present. This calculation relies upon the duration of the various stages being unaffected by the hormonal treatment. This assumption is based on the classic experiments of Clermont & Harvey (1965) in hypophysectomized animals and recently confirmed in the testosterone-suppressed rat (Meistrich *et al.* 1994). As no cell division occurs after the generation of preleptotene spermatocytes, and assuming 100% efficiency in maturation, the HPR of all spermatocyte forms should be identical while the HPR of round spermatids should be fourfold higher. We have confirmed the use of this approach in the testosterone-suppressed rat (McLachlan *et al.* 1994a) and used this approach to isolate the action of testosterone on spermiogenesis (O'Donnell *et al.* 1994). Cell cycle kinetics could also be assessed using nucleotide incorporation ( $^3\text{H}$ ]thymidine, bromodeoxyuridine) but these have not been used in studies of this kind.

#### *Effects of testosterone on spermatogenesis*

Using the testosterone-suppressed model and quantitative germ cell counting methods, a role for testosterone in spermatogonial/spermatocyte development was suggested; however, the principal role was proposed in the transformation of round into elongated spermatids (Sun *et al.* 1990). In more extensive studies examining germ cell populations in the testosterone-suppressed model, we developed these concepts further as outlined below.

**Testosterone and early germ cell development** The acute withdrawal of testosterone produces a distinctive pattern of spermatogenic cell degeneration. When testosterone was removed over the period of a few days by the use of the Leydig cell cytotoxin ethane dimethane sulphonate (EDS), there was a sharp increase in the number of pyknotic nuclei and cytoplasmic vacuoles (both markers of

cellular degeneration) in many stages of the cycle, particularly affecting pachytene spermatocytes and spermatids (Kerr *et al.* 1993). A role for testosterone in prevention of spermatocyte degeneration may be important particularly at stages VII–VIII where degeneration was prevented by concomitant testosterone administration.

In contrast, such degeneration was not seen following the chronic reduction of testicular testosterone levels obtained in the testosterone-suppressed rat; instead spermatogonial and spermatocyte number were reduced to ~60% of normal suggesting a role for testosterone in the maintenance of these cell populations. As the extent of these reductions did not increase with time, the data suggested that spermatogenesis was proceeding at a reduced but nonetheless steady rate (McLachlan *et al.* 1994a). When testicular testosterone levels were partially restored, testis weight and sperm content were restored to ~85% of normal yet spermatogonial number remained significantly reduced. The failure of a high dose of testosterone to normalize spermatogenesis may reflect (i) a requirement of spermatogonia for even higher testosterone levels or (ii) the absence of LH which results in the loss of Leydig cell products other than testosterone that are essential for spermatogenesis, as suggested by studies using EDS (Sprando *et al.* 1990).

**Testosterone and spermiogenesis** The role of testosterone in spermiogenesis has been studied in the testosterone-suppressed rat model by determining the HPR of round spermatids during testosterone withdrawal and replacement (O'Donnell *et al.* 1994). The conversion of steps 1 to 7 round spermatids was maintained in the presence of low testosterone but the conversion of step 7 to 8 spermatids proceeded at only 15% of its normal efficiency. Furthermore, adhesion of round spermatids at this stage appeared to be lost, resulting in their sloughing into the lumen and appearance in the epididymis (O'Donnell *et al.* 1995). When testosterone levels were restored with a high dose of testosterone, the conversion of step 7 to 8 spermatids was normalized within 4 days.

The basis of the testosterone dependency of spermatid/Sertoli cell adhesion may involve effects on the Sertoli cell cytoskeleton. Normally a junctional area, termed the ectoplasmic specialization (ES), develops between Sertoli cells and round spermatids at the onset of the elongation process. The ES (or associated elements) are probably involved in the attachment of round spermatids. In the absence of testosterone, either *in vivo* or *in vitro*, this junction is disrupted (Cameron & Muffly 1991, Muffly *et al.* 1994). Consequently testosterone withdrawal may lead to loss of spermatid adhesion, thereby precluding their further maturation. In a recent study, the testosterone deficiency which followed the destruction of Leydig cells by EDS in pubertal rats resulted in the disruption of the ES and sloughing of round spermatids (Cameron *et al.* 1993).

Based on Sertoli cell–spermatid co-culture experiments, the same authors proposed that FSH is responsible for the alignment of the actin and vinculin filaments of the Sertoli cell cytoskeleton which are needed for this testosterone-dependent adhesion (Muffly *et al.* 1994).

Further studies are required to establish the testosterone-dependent processes involved, especially the involvement of cell adhesion proteins known to exist within the seminiferous epithelium, such as members of the integrin and cadherin families. Both mRNA and immunostaining for N-cadherin have been found in spermatocytes and round spermatids (MacCalman *et al.* 1993) and recently  $\alpha 6\beta 1$  integrin was shown to be localized to the region of elongating spermatid heads (Salanova *et al.* 1995). The regulation of these cell adhesion proteins during manipulation of testosterone levels *in vivo* has not been reported.

**Testosterone–androgen receptor interaction and postreceptor events** Little is known of the mechanism of testosterone action on spermatogenesis beyond the localization of androgen receptors (ARs) on Sertoli, peritubular myoid and Leydig cells (Mulder *et al.* 1975). Most data suggest that the AR is not present on germ cells (Bremner *et al.* 1994); however, immunocytochemical evidence for the presence of ARs in spermatids was recently reported (Vornberger *et al.* 1994). This discrepancy may result from differences in the AR peptides used to generate the antisera or in the immunocytochemical procedures. Pending the confirmation of AR protein in spermatids, the promotion of spermatogenesis by testosterone is generally thought to be mediated through the Sertoli cell. AR expression shows stage-specificity being most abundant in Sertoli cells around stages VII (Bremner *et al.* 1994), a finding consistent with previous data relating to the hormonal sensitivity of stages VII–VIII of the rat spermatogenic cycle (Parvinen 1982).

There are several aspects of testicular testosterone action which are unclear. First, while many authors maintain that testosterone is the androgen responsible for the stimulation of spermatogenesis, the evidence for this is far from conclusive. The affinity of dihydrotestosterone (DHT) for the AR is twice that of testosterone and, in addition, testosterone shows a fivefold faster dissociation rate compared with DHT (Grino *et al.* 1990, Zhou *et al.* 1995). However, because of the relative abundance of testosterone in the normal testis (about five times greater than DHT), it is likely that testosterone is the predominant ligand for the AR (Wright & Frankel 1979). The reported failure of  $5\alpha$ -reductase inhibitors to influence spermatogenesis must be viewed with caution as the blockade of the testicular enzyme was incomplete as evidenced by the readily detectable levels of DHT remaining (George *et al.* 1989). DHT may play a part in normal spermatogenesis but become even more important when testicular

androgen levels are experimentally reduced. Certainly, DHT can restore spermatogenesis at an intratesticular level about half that for testosterone, in keeping with its higher receptor affinity (Chen *et al.* 1994).

The second issue relates to the fact that testicular testosterone levels are vastly higher than the calculated  $K_d$  of the AR *in vitro*, meaning that the receptor should theoretically be saturated at all times and not regulated in the usual way by changes in ligand testosterone concentrations. These high intratesticular levels appear essential for spermatogenesis. Certainly when the testicular testosterone level in rats is reduced to <5% of normal, spermatogenesis is interrupted, even though testosterone is still present at ~10 nmol, i.e. at or above the  $K_d$  for the receptor. In rats, testicular testosterone levels of 50 nmol (~20% of normal) are required for spermatogenesis, a level markedly higher than for other androgen-dependent tissues. The actual level of testosterone is critical (Sun *et al.* 1989, Zirkin *et al.* 1989), as a twofold increase in testosterone dose results in a >20-fold increase in sperm output.

In the absence of any known difference in the AR, why does the testis require a testosterone dose which in peripheral tissues, e.g. the prostate, is clearly supraphysiological? Some aspects of the interaction between testosterone and the AR may differ in the testis as might the interaction of this complex with the DNA. The activation of androgen-dependent pathways involves receptor dimerization, interaction with heat shock proteins and transcription factors, and testis-specific differences in these factors may account for the high testosterone requirement of the testicular AR.

The post-receptor events involved in the stimulation of spermatogenesis by testosterone are unknown. As speculated above, testosterone may affect elements of Sertoli cell structure/function which are necessary for spermiogenesis. Sertoli cell fluid production is principally regulated by androgens (Jegou *et al.* 1983), an action no doubt important to germ cell development. It seems likely that other testosterone-induced proteins specifically involved in the promotion of spermatogenesis exist; however, these have proven elusive. Sharpe *et al.* (1992) have shown that androgen depletion markedly reduced the level of protein synthesis of tubules at stage VI–VII of the cycle (but not at other stages), and identified several candidate proteins susceptible to androgen regulation using two-dimensional gel electrophoresis. Testosterone stimulates Sertoli cell secretion of some proteins (e.g. androgen-binding protein) but inhibits the production (e.g. plasminogen activator) or gene expression of others (e.g. nerve growth factor); however, the relevance of these changes to testosterone action on spermatogenesis has not been established.

#### *Effects of FSH on spermatogenesis*

The specific sites of FSH action on the spermatogenic process are also unclear. While the use of impure

gonadotrophin preparations seriously flawed earlier work, the availability of recombinant hormone now permits specific, if only short-term, replacement studies. The lack of recombinant rat gonadotrophin preparations prevents the necessary long-term experiments required by the long duration of the spermatogenic cycle in the rat (~50 days).

Studies using the GnRH antagonist-treated (Sinha-Hikim & Swerdloff 1993) or hypophysectomized rat in short-term maintenance models (Bartlett *et al.* 1989, Kerr *et al.* 1992, Russell *et al.* 1993) suggested that FSH supports germ cell development up to the round spermatid stage but, in the absence of testosterone, the completion of the final stages of spermiogenesis was not achieved (Bartlett *et al.* 1989, Kerr *et al.* 1992). Recombinant FSH treatment of the GnRH antagonist-treated rat resulted in the maintenance of normal type B spermatogonial and preleptotene spermatocyte number for up to 4 weeks while pachytene spermatocyte and round spermatid number were only partially and transiently maintained (Sinha Hikim & Swerdloff 1995).

Using recombinant FSH to restore spermatogenesis in the GnRH-immunized rat model, germ cell numbers were determined using the optical disector stereological approach (McLachlan *et al.* 1995) in order to define the cellular site(s) of FSH action. Following GnRH immunization, all germ cell types were significantly reduced (spermatogonia ~46%→round spermatids ~5% of normal). FSH treatment increased the numbers of all cells types prior to elongated spermatids. An increase in type A spermatogonial number to 65% of normal was consistent with other data, suggesting this as a site of FSH action. On the other hand, the several-fold increase in early and late pachytene spermatocyte and round spermatid number seen within 1 week suggest an increase in cell survival. At no stage did FSH increase elongated spermatid numbers above 1% of control levels. In both GnRH-immunized and FSH-treated animals, the mitotic activity of spermatogonia, determined by the incorporation of the bromodeoxyuridine, was unaltered. It can be concluded that recombinant FSH partially restored spermatogenesis by increasing the number of spermatogonia and by promoting subsequent maturational steps, including the meiotic reduction of late pachytene spermatocytes to round spermatids. An increase in germ cell survival is most likely a major factor in these changes. This is in agreement with a recent study which showed that recombinant FSH could partially prevent the degeneration of germ cells in adult rats induced by hypophysectomy (Russell *et al.* 1993). With the increasing understanding of the process of apoptosis (programmed cell death), it remains to be seen whether the action of FSH is largely an 'anti-apoptotic' one, in a similar way to testosterone's action on the prostate. Both testosterone and FSH have been reported partially to prevent apoptotic changes in immature rats following hypophysectomy (Tapanainen *et al.* 1993).

Recently apoptotic DNA changes were also reported to be stage-specific in adult rats, affecting primarily spermatocytes in stages I and XII–XIV rather than at stage VIII (Billig *et al.* 1995). Apoptotic changes were reported in spermatocytes and round and elongated spermatids at stages VII–VIII following GnRH antagonist treatment (Sinha-Hikim *et al.* 1995).

While it is clear that FSH is involved at multiple sites from spermatogonia (i.e. entry into the cycle) onwards by promoting germ cell survival and transition between cell types up to round spermatids, it should be noted that spermatid elongation was not restored by FSH, indicating the need for additional factor(s), most likely testosterone as described above.

#### *FSH, testosterone and human spermatogenesis*

In non-human primates and man, there is good evidence that FSH is required for spermatogenesis. In monkeys, a 50% reduction in testis size and a substantial reduction in sperm output was achieved by active and passive immunization against FSH (Srinath *et al.* 1983). In other studies, administration of highly purified FSH to GnRH antagonist-treated monkeys fully maintained spermatogonial numbers and those of spermatocytes and spermatids at 50% of control (Weinbauer *et al.* 1991). Finally, in man, a role of FSH has been well demonstrated by the studies of Matsumoto *et al.* (1983) who showed that FSH treatment could partially restore sperm counts in men in whom spermatogenesis was suppressed by testosterone-induced cessation of gonadotrophin secretion. The quantitative restoration of sperm counts could only be achieved by combined gonadotrophin treatment using FSH and human chorionic gonadotrophin (as an LH substitute) (Matsumoto *et al.* 1986), establishing the need for both hormones.

#### *Testosterone and FSH co-operativity*

From the above it can be argued that particular roles exist for FSH and testosterone in the spermatogenic process (see Fig. 1). For example, FSH is involved in increasing spermatogonial number and the maturation of spermatocytes, including meiosis. An essential role for testosterone in spermatid maturation is apparent as even high doses of FSH are unable to complete this vital step. Nonetheless there are many reports suggesting that FSH and testosterone act co-operatively and indicate that a lower dose of either is equally effective when the other is present (Bartlett *et al.* 1989, Sun *et al.* 1989, 1990, Sinha-Hikim & Swerdloff 1994). This view has led to the speculation that they may have common post-receptor pathways of action (Russell *et al.* 1993), evidence for which is still lacking. There are several points at which this type of co-operativity between FSH

and testosterone may exist. The first relates to the Sertoli cell cytoskeleton and associated Sertoli–germ cell junctions. In regard to the action of testosterone on spermiogenesis, FSH influences the distribution of cell structural proteins, f-actin and vinculin in hypophysectomized testosterone-treated rats (Muffly *et al.* 1994). These structural proteins may help determine the capacity of Sertoli cells to bind spermatids under the influence of testosterone (see above). *In vitro* evidence supports the synergistic role of FSH on testosterone-mediated spermatid binding (Cameron & Muffly 1991). Similar cytoskeletal effects of FSH may be crucial in other Sertoli–germ cell interactions.

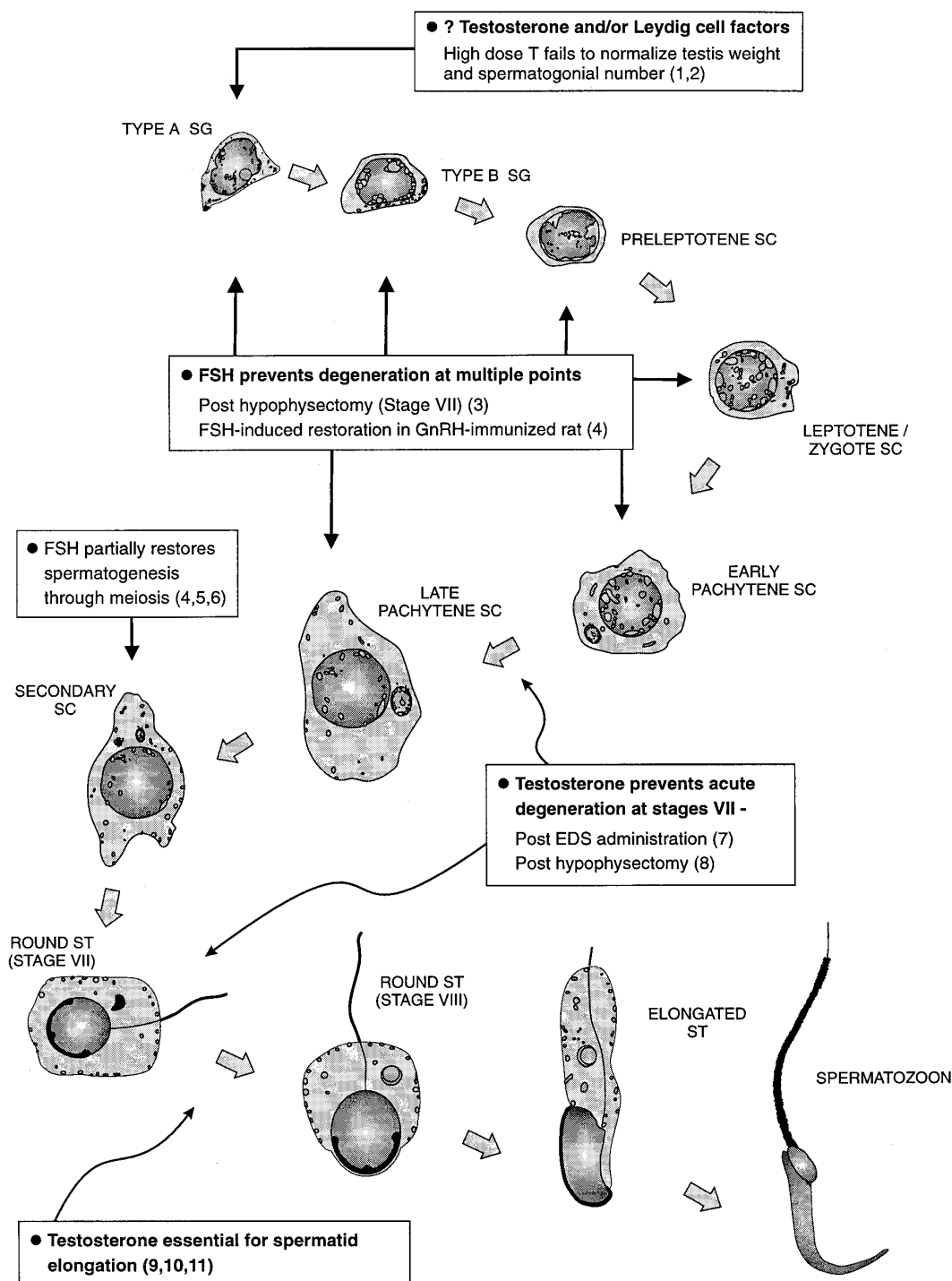
Secondly, at the receptor level, FSH may synergize with testosterone by stimulating the synthesis of the AR, based on *in vitro* studies in which FSH resulted in a two- to threefold increase in AR number (Verhoeven & Cailleau 1988).

FSH treatment of hypophysectomized rats promoted the effect of testosterone on spermiogenesis and was associated with increased testicular content of androgen-binding protein in the testis and epididymis (Huang *et al.* 1991) and, through this mechanism, it was suggested therefore that FSH has a role in facilitating the transport and localization of testosterone within Sertoli cells. In the absence of identified second messengers for testosterone and FSH, one can only speculate as to the roles of FSH and testosterone (individual or synergistic) in the production of Sertoli cell growth factors involved in spermatogenesis.

#### *Conclusion*

Notwithstanding differences in experimental design and animal physiology, there is overall good evidence for roles for both testosterone and FSH in spermatogenesis. For FSH, actions include stimulation of Sertoli cell division, maturation, secretory capacity and cytoskeletal arrangement. In terms of germ cells, these actions include stimulation of spermatogonial division and meiosis and perhaps an anti-apoptotic action on spermatogonia and spermatocytes. For testosterone, a prominent role in spermiogenesis is clear. Good evidence exists to indicate that synergism occurs between both hormones at and beyond binding to receptors. While some species differences exist in the extent of this synergism, the basic proposition that both testosterone and FSH have normal physiological roles has not been challenged in any substantial way by existing data.

Beyond the classic studies of Matsumoto *et al.* (1983, 1986), the extension of studies into the human have been very limited indeed. A better understanding of testosterone action in the human is essential as testosterone is already being proposed as a contraceptive (World Health Organisation Task Force 1990). While it is assumed that



**Figure 1** The effect of testosterone and FSH on the progression of spermatogenic cells in the adult rat are shown. Relevant references for each effect are given. Note that FSH acts predominantly to support spermatogonial (SG) number, to prevent the premature degeneration of SGs and spermatocytes (SCs) and to support meiotic division of pachytene SCs to yield round spermatids (STs). The principal effect of testosterone is to facilitate the progression of round STs (at stages VII–VIII of spermatogenesis) into elongated forms, perhaps by maintaining their attachment to Sertoli cells. There are also several sites of synergism between FSH and testosterone (see text for details). (1, Zirkin *et al.* 1989; 2, McLachlan *et al.* 1994a; 3, Russell *et al.* 1993; 4, McLachlan *et al.* 1995; 5, Kerr *et al.* 1992; 6, Bartlett *et al.* 1989; 7, Kerr *et al.* 1993; 8, Russell & Clermont 1977; 9, Sun *et al.* 1990; 10, O'Donnell *et al.* 1994; 11, Cameron & Muffly 1991.) (Cell morphologies modified after Russell *et al.* 1990.)

testosterone acts in a similar way to that described in the testosterone-suppressed rat, there are no data on the physiological or structural changes resulting from testosterone withdrawal on the human testis. For example, in ~35% of men, azoospermia is not produced; however, the basis of this failure is not known but appears to result from intrinsic differences in the spermatogenic process between individuals (Handelsman *et al.* 1995). Clearly the therapeutic application of testosterone as a contraceptive is far ahead of our understanding of the basic physiology. Future studies on the impact of testosterone withdrawal on human spermatogenesis require quantitative assessments of its effects on germ cell populations.

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