Progress Towards a Male Contraceptive

Edited by
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Progress Towards a Male Contraceptive

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Series Preface

Human reproductive endocrinology is a field that has seen great advances in knowledge in recent years. This has been based initially upon refinements in laboratory techniques which have led to an expansion of our understanding of the biochemical and physiological mechanisms underlying the control of reproduction in man and other animals, and subsequently to improvements in the diagnosis and treatment of patients.

This series of books is based upon a series of annual one-day symposia held at the Institute of Obstetrics and Gynaecology in the University of London. The aim is to cover an individual topic within the broad subject of reproductive endocrinology, giving the experimental background where appropriate, and to detail the current application to human endocrinology and clinical medicine.

September, 1981 S.L. JEFFCOATE

Preface

Population control on a world-wide scale will continue to be an important topic for the foreseeable future. In the past 30 years basic research on the control of the female reproductive cycle and parallel pharmaceutical developments have led to progressively more effective and safer chemical methods of fertility control in women. In the case of the male, however, a safe and effective pill is still far from available. There are several reasons for this contrast: an attitude, prevalent in many societies, that contraception is the responsibility of women; a poor understanding of the factors controlling male fertility; and a different climate in pharmaceutical research in which the development and introduction of new drugs is considerably more expensive than it was 30 years ago.

This book has been designed to present the most recent developments in chemical methods of male contraception and to indicate possible future trends. Current understanding of the factors affecting male fertility are described and the potential contraceptive approaches are comprehensively and realistically evaluated. It will be of interest to both clinicians and scientists working in the field of human reproduction and contraception and also to those concerned with pharmaceutical developments and the administration of family planning services.

It will be seen that progress towards a male contraceptive that is effective and safe, cheap and acceptable, is rather slow but we hope that this volume summarizes what has been achieved by late 1981 and provides some pointers for the future.

London, January, 1982

S.L. JEFFCOATE
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CHAPTER 1

Regulation of spermatogenesis and possible sites for contraceptive action

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1. NORMAL SPERMATOGENESIS

Any discussion of the control of spermatogenesis in mammals must begin with a summary of the main features of normal spermatogenesis. The types of germinal cells found in the testis (see Table 1) are the same in mammals as in lower animals (see Pilsworth and Setchell, 1981) in many of which spermatogenesis proceeds in cysts in which all the germ cells are at the same stage of development (Figure 1). In mammals, on the other hand, five or six different generations of germ cells can be found associated in one section of a seminiferous tubule (Figure 2), although, as in the lower animals, the germ cells develop in close association with special somatic cells—the Sertoli cells

Table 1. Types of germ cells found in the Mammalian Testis

Cell type	*Division*
Stem spermatogonia (A_S or A_0)	Mitosis, not synchronized with other events in the cycle
Spermatogonia (A_1, A_2, A_3, A_4, In and B)	Mitosis, synchronized with particular stages of the cycle
Primary spermatocytes (preleptotene, leptotene, zygotene, pachytene, diplotene)	First meiotic division, at a particular stage of the cycle
Secondary spermatocytes	Second meiotic division, at a particular stage of the cycle
Spermatids	Do not divide

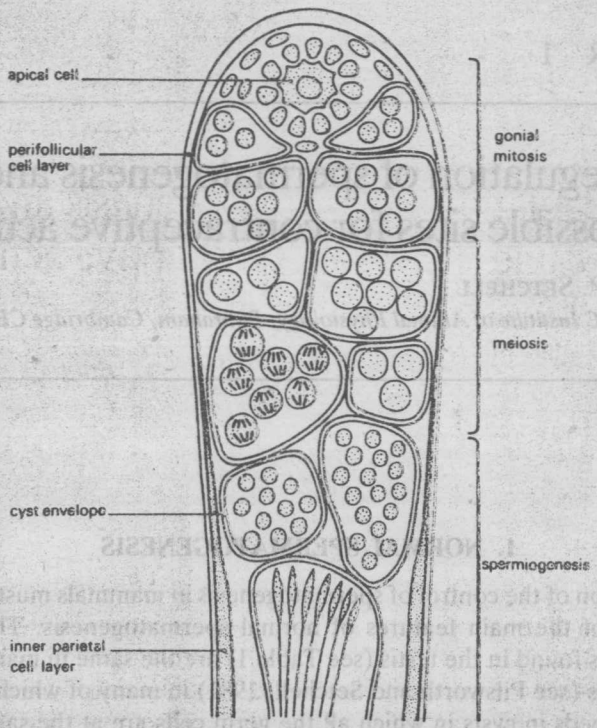

Figure 1. A diagram illustrating spermatogenesis in an insect. The germ cells and the cells forming the cyst envelope arise by division of the cells near the germ cells, the spermatogonia, which undergo a series of mitotic divisions while the cyst is in the zone marked 'gonial mitosis'. Then, as the cyst is moved along the testis by the formation of new cysts, the germ cells become spermatocytes and enter meiosis. After meiosis, the spermatids develop into spermatozoa which are finally shed by rupture of the cyst. Reproduced from Szöllösi and Marcaillou (1977)

in mammals and the cells forming the wall of the cyst in the lower animals. Cytoplasmic bridges between germ cells at a similar stage of development are also a feature of spermatogenesis in many, if not all, classes of animals (Dym and Fawcett, 1971) and this means that the germ cells develop not as individual cells but as clones of up to several hundred cells in mammals in the later stages.

A most remarkable characteristic of mammalian spermatogenesis is that, at any point in the wall of a seminiferous tubule, certain germ cells are only ever found in association with certain other germ cells, and never with others (Figure 3). This is presumably because from the first synchronized development of the A_1 spermatogonia, the development of all the germ cells proceeds always at the same rate as that of other cells in that segment of the tubule, so

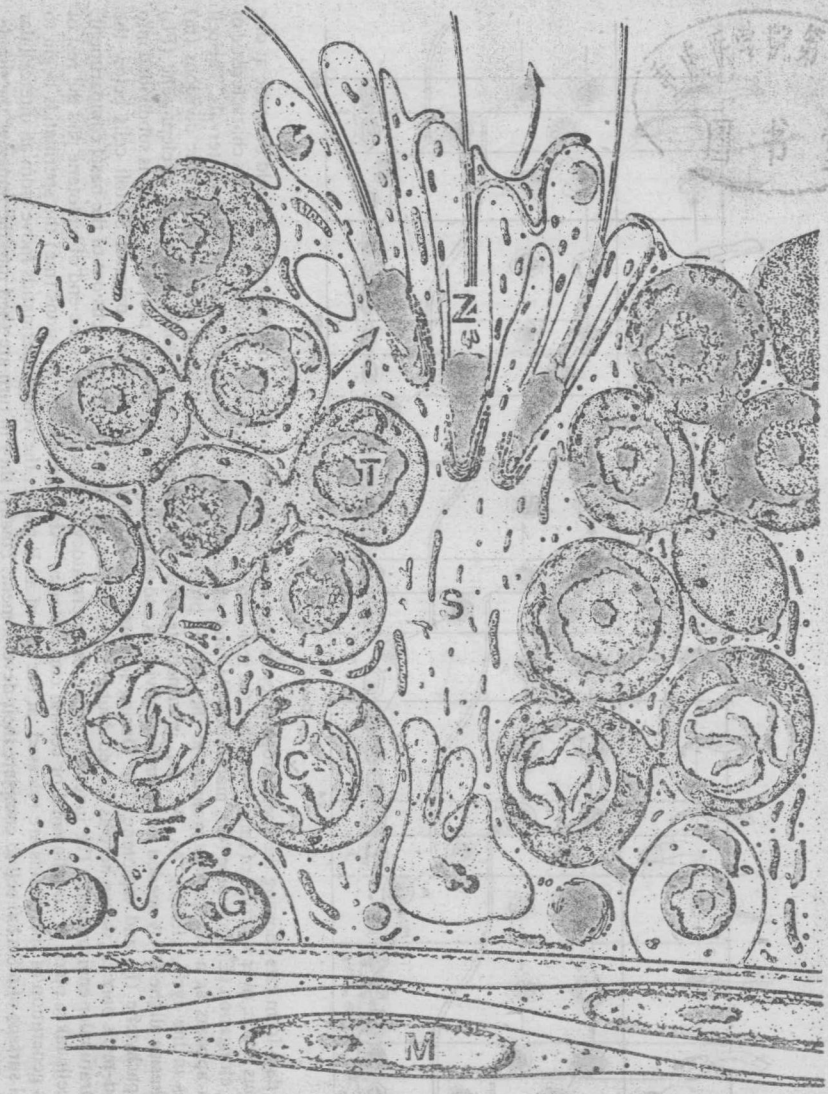

Figure 2. A diagram illustrating spermatogenesis in a mammal. The germ cells, spermatogonia (G), spermatocytes (C), early spermatids (T) and late spermatids (Z) are all closely associated with the same somatic Sertoli cells (S), and the whole tubule is surrounded by a second type of somatic cell, the myoid cells (M). Reproduced from Fawcett (1974)

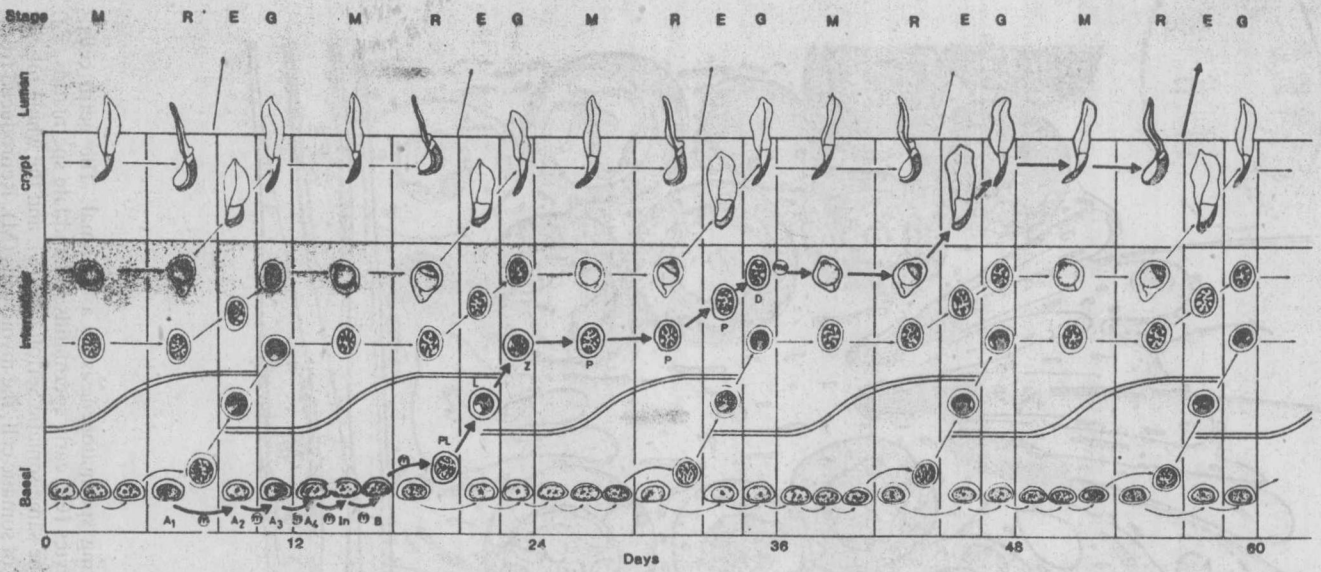

Figure 3. A diagram based on data from rats showing the spermatogenic cycle that would be seen if a series of observations could be made at one point in the wall of a seminiferous tubule over a period of time. Note that the scale across the bottom is in days. The cell associations characteristic of the four stages of the cycle, i.e. elongation (E), grouping (G), maturation (M) and pre-release (R), are shown recurring at 12-day intervals. The cell chosen for particular attention appears as a A_1 spermatogonium in the first R phase, then develops through a series of mitotic divisions (m) synchronized with other events in the cycle, yielding A_2, A_3, A_4, intermediate (In) and B-type spermatogonia, and finally preleptotene (PL) spermatocytes. These cells synthesize all the DNA needed for the meiotic divisions and then enter the long meiotic prophase, which is subdivided into leptotene (L), zygotene (Z), pachytene (P) and diplotene (D). During leptotene, junctions appear between pairs of Sertoli cells below the spermatocytes to form the blood–testis barrier (shown here as curved double lines) moving the developing germ cells out of the basal compartment, and isolating them from the extratubular environment. The junctions above the spermatocytes then open up at the end of leptotene and the rest of the development of the germ cells takes place in an adluminal or central compartment. After the meiotic divisions (me) the spermatids begin to mature, and when the previous generation of spermatids are released, at the end of an R phase, the young spermatids move from an intercellular position to crypts in the luminal surface of a Sertoli cell to complete their development. Finally, they in turn are shed into the lumen of the tubule. Note that with each recurrence of the same cell association with the passage of one whole cycle, our chosen cell is one generation further advanced. For the origin of the A_1 spermatogonia see Figure 4

that they all transform to the respective next cell type at the same time, and the cell associations of the various stages of the seminiferous cycle are preserved.

Several ways of classifying the stages of the cycle have been suggested, based either on overall cell patterns, or on the appearance of the acrosome in the spermatid. However, I believe that all the schemes are too detailed and complicated for general use, and I would like to suggest a simplified system of four stages, named from the appearance of the most mature spermatids in each cell association. Beginning, for convenience, after the shedding of the previous spermatozoa (although as with all true cycles, there is no real beginning) there is an elongation (E) stage, a grouping (G) stage, a maturation (M) stage and a pre-release (R) stage (Figure 3). Each of these types of spermatids has certain other cell types always in association with them. The stages of this scheme can be easily related to those in the more intricate systems (Table 2), and it has the advantage that, as Parvinen and Vanha-Perttula (1972) have shown, four very similar stages can be recognized under phase contrast in whole live seminiferous tubules of the rat.

It is important not to confuse the spermatogenic cycle—a cycle of changes with time at one point in the tubule—with the spermatogenic wave, which is the reasonably orderly arrangement of stages of the cycle spatially along the tubule at one point in time. In general, one moves from later to earlier stages of the cycle as one moves away from the rete, but this first wave is followed by a series of many further waves of complete sets of stages of the cycle; there are also frequently modulations in the waves, where the order of stages is reversed for a short distance and there is a 'point of reversal' somewhere along the tubule where the two series meet (Perey, *et al.*, 1961). The wave, in contrast to the cycle, is seen only in some species such as rats and bulls and not in man, and is presumably a consequence of the way in which spermatogenesis is established, although its maintenance must depend on the synchronized cyclical development of germ cells. Even the stages of the cycle were difficult to recognize in the human testis in which the cell associations cover only small areas of the wall of the tubule, not always extending even right round the tubule. This is also in contrast to laboratory and domestic animals in which whole cross-sections and indeed considerable lengths of tubule are usually found at the same stage of the cycle.

The relative duration of various stages of the cycle can be determined by counting how frequently the stages occur in a large number of randomly selected cross-sections. The length of one whole cycle can be estimated from the rate of development of tritiated thymidine-labelled germ cells, the last synthesis of DNA taking place in the preleptotene stage of the meiotic prophase. The development of a spermatozoon from the first synchronized spermatogonial mitosis takes four complete cycles (Figure 3). In man each cycle lasts about 16 days (so that the whole process takes about 64 days) and

Table 2. The relationship between the four suggested stages in the spermatogenic cycle, and other published schemes. For details and references see text. The height of each panel represents its relative duration, as a portion of one whole cycle between two successive releases of spermatozoa

<table>
<tr><th>Suggested simplified scheme</th><th>Parvinen's description of tubule under phase contract</th><th>Huckins' simplified scheme</th><th>Leblond and Clermont's scheme (Perey et al., 1961)</th><th>Von Ebner, Curtis, Roosen-Runge, and Ortavant's scheme</th><th></th></tr>
<tr><td rowspan="2">Elongation (E)</td><td rowspan="2">Pale</td><td rowspan="3">2</td><td>IX</td><td rowspan="2">1</td><td rowspan="2">← Release of sperm</td></tr>
<tr><td>X</td></tr>
<tr><td rowspan="2">Grouping (G)</td><td>XI</td><td rowspan="2">2</td><td></td></tr>
<tr><td rowspan="2">Pale spots</td><td>XII</td><td rowspan="2">3</td><td rowspan="2"></td></tr>
<tr><td rowspan="2">3</td><td>XIII</td><td rowspan="2">} Meiosis</td></tr>
<tr><td rowspan="3">Maturation (M)</td><td>XIV</td><td>4</td></tr>
<tr><td rowspan="2">4</td><td rowspan="2">I</td><td rowspan="3">5</td><td rowspan="2"></td></tr>
<tr><td rowspan="2">Dark spots</td><td rowspan="3">5</td><td>II</td></tr>
<tr><td>III</td><td rowspan="3">6</td><td rowspan="2"></td></tr>
<tr><td>IV</td></tr>
<tr><td rowspan="2">6</td><td rowspan="2">V</td><td rowspan="2"></td></tr>
<tr><td rowspan="2">Pre-release (R)</td><td rowspan="2">Dark centre</td><td rowspan="2">1</td><td>VI</td><td rowspan="2">7</td><td rowspan="2"></td></tr>
<tr><td rowspan="2">VII</td><td rowspan="2">8</td></tr>
<tr><td></td><td>VIII</td><td>← Release of sperm</td></tr>
</table>

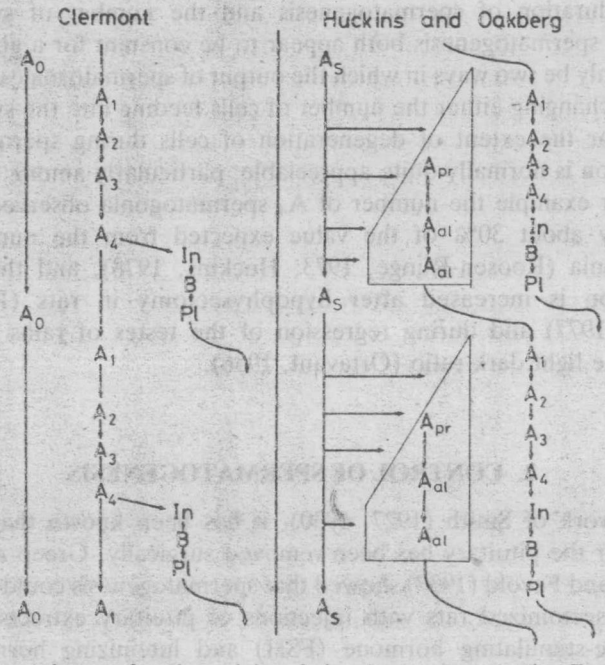

Figure 4. Two schemes for the origin of the A_1 spermatogonia. For details and references see text

the length of the cycle appears to be quite constant for any species (see Setchell, 1978).

The origin of the A_1 spermatogonia is still the subject of some controversy (Figure 4). Clermont (1962, 1972), Clermont and Bustos-Obregon (1968), and Clermont and Hermo (1975) believe that they originate by division of the A_4 spermatogonia, which can yield either A_1 spermatogonia or differentiating In (intermediate) spermatogonia; these authors also recognize a population of stem cells (A_0 spermatogonia) which only contribute significantly to the pool of dividing cells if the latter are depleted, e.g. after X-irradiation. On the other hand, Huckins (1971), Oakberg (1971) and de Rooij (1973) [see also Huckins (1978) and Huckins and Oakberg (1978)] believe that the stem cells (which they call A_S spermatogonia) divide continually, but their divisions are not synchronized with other events in the spermatogenic cycle. When the cytoplasmic connections are broken at the end of a division, the daughter cells remain as stem cells; if the bridges persist, the pairs (A_{pr}), or groups (A_{al}) of cells if further divisions ensue, feed into the cycle towards the end of the maturation (M) phase [stage V by the Leblond and Clermont scheme, (Perey *et al.*, 1961)].

As the duration of spermatogenesis and the number of synchronized divisions in spermatogenesis both appear to be constant for a given species, there can only be two ways in which the output of spermatozoa can be varied, namely by changing either the number of cells feeding into the system at the beginning or the extent of degeneration of cells during spermatogenesis. Degeneration is normally quite appreciable, particularly among the spermatogonia, for example the number of A_4 spermatogonia observed in normal rats is only about 30% of the value expected from the number of A_1 spermatogonia (Roosen-Runge, 1973; Huckins, 1978), and the extent of degeneration is increased after hypophysectomy in rats (Russell and Clermont, 1977) and during regression of the testes of rams induced by changing the light:dark ratio (Ortavant, 1956).

2. CONTROL OF SPERMATOGENESIS

Since the work of Smith (1927, 1930), it has been known that the testes regress after the pituitary has been removed surgically. Greep *et al.* (1936) and Greep and Fevold (1937) showed that spermatogenesis could be restored in hypophysectomized rats with injections of pituitary extracts containing both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (so-called from their effects in female animals); they also showed that FSH alone stimulated the tubules without affecting the androgen-dependent accessory glands, while LH stimulated the Leydig cells to produce androgens which were also important for some tubular functions. However, these earlier studies were done with relatively impure hormone preparations. More recent experiments with highly purified material have shown that both FSH and LH are needed to maintain growth of the testis after hypophysectomy in immature rats (Lostroh, 1969; Courot, *et al.*, 1971) and ram lambs (Courot and Ortavant, 1972), and that FSH stimulates the incorporation of tritiated thymidine into spermatogonia and preleptotene spermatocytes in the testes of immature rats, while LH is without effect (Ortavant *et al.*, 1972).

However, these results are hard to reconcile with the observation that, in the adult rat, large doses of testosterone administered immediately after hypophysectomy prevent regression of the testes (Walsh *et al.*, 1933, 1934; Nelson and Gallagher, 1936; Nelson and Merckel, 1937; Ahmad *et al.*, 1973, 1975). This treatment could not reinitiate spermatogenesis once regression of the testes had occurred in the rats, but in squirrels (Wells, 1942) and rhesus monkeys (Smith, 1944) it appeared to do so. Even more puzzling is the observation that in guinea pigs some spermatogenesis persists for quite a long time after hypophysectomy (Allanson *et al.*, 1935) although in this species also, testosterone appears to have some protective effect (Cutuly, 1941).

On the other hand, in adult rams, testosterone had no effect on post-

hypophysectomy regression of the testis, even when given in doses which maintained normal concentrations of this steroid in rete testes fluid (Courot *et al.*, 1979). There is also an important species difference in the stage at which spermatogenesis is arrested. In hypophysectomized rats, spermatogenesis is arrested at the pachytene spermatocyte stage, although there is some reduction in the numbers of B spermatogonia formed [see Setchell (1978) for review of earlier observations; Chowdhury, 1979]. In rams, no spermatocytes are formed once the testis has regressed (Courot *et al.*, 1979), and in primates (Smith, 1944) including man, the post-hypophysectomy appearance of the testis appears to resemble that in sheep. In hypophysectomized men, the regressed testes can be restored by treatment with FSH-like human menopausal gonadotrophin (hMG) plus human chorionic gonadotrophin (hCG) (Gemzell and Kjessler, 1964; MacLeod *et al.*, 1966; MacLeod, 1970; Mancini *et al.*, 1968), and spermatogenesis restored by this form of treatment in hypophysectomized or hypogonadotrophic men can be maintained with hCG alone (Johnsen and Christiansen, 1968; MacLeod, 1970; Johnsen, 1978). There is also evidence that in men spermatogenesis suppressed by testosterone can be restored by additional treatment with hCG in the presence of continuing undetectable levels of FSH (Bremner *et al.*, 1980; Matsumoto *et al.*, 1981). This could be due to the hCG producing higher concentrations of testosterone locally in the testis; the authors report only peripheral serum testosterone concentrations, and they found higher values with hCG + testosterone than with testosterone alone. Alternatively, hCG may have some FSH-like activity (Northcutt and Albert, 1970) although usually it is considered to be predominantly, if not exclusively, LH-like in its actions.

As well as surgical hypophysectomy, immunological suppression of gonadotrophin secretion, using active or passive immunization against luteinizing hormone releasing hormone (LHRH) has been shown to result in atrophy of the testis (see Chapter 3), but restoration of spermatogenesis with gonadotrophins or testosterone in animals treated in this way does not seem yet to have been attempted. This approach would have the obvious advantage of using animals with normal levels of the other pituitary hormones such as growth hormones, TSH, ACTH and prolactin, and it is to be hoped that these experiments will soon be done.

Another approach has been to withdraw each gonadotrophin separately using either passive or active immunization against the respective hormone. The earlier studies, reviewed by Setchell and Edwards (1975), showed that LH was certainly required for normal testis function, but the evidence for a role for FSH was equivocal. More recent studies in rats have confirmed the importance of FSH in the initiation of spermatogenesis in immature rats (Raj and Dym, 1976; Dym *et al.*, 1977; Chemes *et al.*, 1979a), but no effects could be demonstrated in adults (Dym *et al.*, 1979; Davies *et al.*, 1979). In contrast, passive immunization against FSH in adult bonnet (Murty *et al.*, 1979) and

rhesus monkeys (Wickings *et al.*, 1980; see also Chapter 4) led to reductions in spermatogenesis.

It is obvious that there are significant species differences in the role of FSH and also important differences in at least some species in its importance in the initiation of and the maintenance of spermatogenesis. It is also important to distinguish between a permissive role in allowing a process to proceed and a regulatory role, which implies quantitative control. No-one, to my knowledge, has ever produced evidence that levels of FSH quantitatively *control* spermatogenesis in adult animals, and no-one has ever produced evidence that the output of spermatozoa or the weight of the testis (sperm output per gram of testis seems to have an upper limit for a given species) can be increased in adult animals by injections of FSH or any other hormone. However, in immature hypophysectomized hemicastrated hCG-treated rats, the growth of the remaining testis did show a good dose–response to three levels of dosage with ovine FSH or rat pituitary (Gans and van Rees, 1966). The lack of effect of hormones or spermatogenesis in adult animals is supported by the failure of hCG + hMG treatment to influence sperm concentrations in the semen of oligozoospermic men (Sherins, 1974).

Some encouraging evidence for a potential site for control of spermatogenesis comes from studies on the effects of hemicastration. In adult rats, this does not lead to hypertrophy of the remaining testis (Setchell and Waites, 1972) and there is no effect on plasma FSH (Howland and Skinner, 1975). In seasonally breeding sheep, hypertrophy is only seen in the next breeding season, although regression of the remaining testis at the end of the breeding season is retarded (Hochereau-de Reviers, *et al.*, 1977). In contrast, in immature rats there is an appreciable rise in plasma FSH after hemicastration at 10 days of age, and compensatory hypertrophy of the remaining testis has occurred by 30 days after hemicastration at 5, 10, 15 or 20 days but not after hemicastration at 45 or 75 days of age (Cunningham *et al.*, 1978). This is due mainly to an increase in the numbers of Sertoli cells per testis (Hochereau-de Reviers, 1975).

In immature sheep, hemicastration at one week of age produces an immediate rise in serum FSH levels with no difference from control in levels of LH or testosterone. These changes in FSH are followed by compensatory hypertrophy of the remaining testis and advancement of the beginning of spermatogenesis (Walton *et al.*, 1978, 1980). In ram lambs hemicastrated when they were 6, 12 or 16 weeks of age, compensatory hypertrophy of the testis also occurs, but in this case associated with changes in LH, not in FSH (Land and Carr, 1975; Hochereau-de Reviers *et al.*, 1980). The total number of A_0 spermatogonia per testis was also increased, as were the daily productions of leptotene spermatocytes and round spermatids, but there was no change in the daily production of A_1 spermatogonia (Hochereau-de Reviers *et al.*, 1980), suggesting that the yield of the spermatogonial divisions