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Review

Hormonal control of germ cell development and spermatogenesis

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ABSTRACT

Spermatogenesis is completely dependent on the pituitary hormone follicle-stimulating hormone (FSH) and androgens locally produced in response to luteinising hormone (LH). This dual control has been known since the 1930s and 1940s but more recent work, particularly using transgenic mice, has allowed us to determine which parts of the spermatogenic pathway are regulated by each hormone. During the first spermatogenic cycle after puberty both FSH and androgen act to limit the massive wave of germ cell apoptosis which occurs at this time. The established role of FSH in all cycles is to increase spermatogonial and subsequent spermatocyte numbers with a likely effect also on spermiation. Mice lacking FSH or its receptor are fertile, albeit with reduced germ cell numbers, and so this hormone is not an essential regulator of spermatogenesis but acts to optimise germ cell production Androgens also appear to regulate spermatogonial proliferation but, crucially, they are also required to allow spermatocytes to complete meiosis and form spermatids. Animals lacking androgen receptors fail to generate post-meiotic germ cells, therefore, and are infertile. There is also strong evidence that androgens act to ensure appropriate spermiation of mature spermatids. Androgen regulation of spermatogenesis is dependent upon action on the Sertoli cell but recent studies have shown that androgenic stimulation of the peritubular myoid cells is also essential for normal germ cells development. While FSH or androgen alone will both stimulate germ cell development, together they act synergistically to maximise germ cell number. The other hormones/local factors which can regulate spermatogenesis include activins and estrogens although their role in normal physiological regulation of this process needs to be more clearly established. Regulation of spermatogenesis in primates appears to be similar to that in rodents although the role of FSH may be greater. While our knowledge of hormone function during spermatogenesis is now well developed we still lack understanding of the mechanisms by which these hormones act to regulate this process.

19 **Contents**

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1. Introduction

Development and maintenance of spermatogenesis in the adult is critically dependent upon the actions of follicle-stimulating hormone (FSH) and androgen. This has been clear since the early pioneering studies by Smith, Greep and others in the 1920s and 1930s who used hypophysectomy and hormone replacement techniques to identify the pituitary and testicular factors involved [1–3]. In the intervening years hormone ablation/replacement techniques have become more refined but the fundamental story remains the same and most recently it has been shown that in animals lacking receptors for both follicle-stimulating hormone (FSH) and androgen only 3% of normal germ cell numbers are present at day 20 [4]. Improved techniques for hormone ablation/replacement and the development of transgenic models mean that we are now able to identify the stages of spermatogenic development and maintenance that are affected by each hormone. In addition we can show how these hormones interact and we are starting to understand the cellular mechanisms that regulate hormone action on spermatogenesis.

The Sertoli cells are critical for all aspects of spermatogenesis from maintenance of the spermatogonial stem cell niche to the final process of spermiogenesis. Indeed, recent studies have shown that specific ablation of a single protein (Dicer) from the Sertoli cells will lead to loss of all germ cell types in the adult testis [5]. Both FSH and androgen act to regulate spermatogenesis through receptors on the Sertoli cells and this appears to be the only way that FSH directly influences the process. Androgens also act through peritubular myoid cells (PTMC) to maintain spermatogenesis [6] although this effect is probably mediated via the Sertoli cells. Total germ cell numbers in the adult testis are dependent upon Sertoli cell numbers [7] and, overall, it is clear that the course of spermatogenesis is completely dependent on the development and maturation of the Sertoli cells.

Until recently the main experimental approaches to the study of FSH and androgen action have been hypophysectomy, treatment with a gonadotrophin-releasing hormone (GnRH) agonist or antagonist (each followed by hormone replacement), injection of ethanedimethane sulfonate (EDS) (which ablates Leydig cells) or immunization against the hormone or receptor. Each has significant drawbacks: hypophysectomy will clearly disrupt other hormones (including LH and, thereby, testosterone), GnRH antagonists are not completely effective, particularly against FSH, EDS is a cytotoxin which may have off-target effects while immunization also has limited effectiveness and in many of the older studies is not specific to FSH (12;13). The field is currently, however, in a second period of major progress using mouse models which was started initially by study of the testicular feminised (*tfm*) mouse which lacks functional androgen receptors (AR) [8] and the hypogonadal (*hpg*) mouse which has a severe deficiency in circulating gonadotrophins [9]. Study of these natural mutants was then enhanced by the generation of transgenic mice lacking specific hormones or their receptors either ubiquitously or on specific cell types. We now have available animals lacking FSH-receptors (FSHRKO), androgen receptors (ARKO), androgen receptors specifically on the Sertoli cells (SCARKO) and androgen receptors specifically on the PTMs (PTM-ARKO). These models, however, suffer the weakness that the hormone is lacking throughout development and, therefore, tell us more about the initiation of spermatogenesis than they do about normal maintenance. So far there is only one report of an inducible model of hormone/receptor knockout in the testis [10] and this review is based largely on what we know from non-inducible models and from older, interventionist studies in the rat

2. Gonocyte development

Early gonocyte development in the mouse occurs before formation and functional maturity of the pituitary and so will not be under regulation by the gonadotrophins. This has been confirmed by study of *hpg* and FSHRKO mice [4,11]. The fetal Leydig cells, the source of testicular androgen, start producing testosterone soon after testis formation [12,13] but data from the ARKO mouse shows that germ cell numbers are normal at birth in the absence of androgen action [4]. Similarly, in mice lacking both AR and FSHR gonocyte numbers are normal at birth [4]. Between the day of birth and day 5 in the mouse there is a marked proliferation of the gonocytes [11,14] and the cells migrate from the lumen of the seminiferous tubule to rest on the basement membrane as spermatogonial stem cells (SSC) and spermatogonia [11,15]. In *hpg*, ARKO, SCARKO and FSHRKO mice (and combined FSHRKO.ARKO and FSHRKO.SCARKO mice) there is a significant (~45%) reduction in testicular germ cells at day 5 although germ cell migration is normal [4,11]. This suggests that gonocyte proliferation/survival just prior to SSC development is hormone-dependent although there is redundancy of action between the hormones. Platelet-derived growth factor (PDGF) and NOTCH signalling from the Sertoli cells appear also to be involved in gonocyte proliferation [16,17]. It is possible that FSH and androgen act through secretion of PDGF but gonocyte migration and differentiation are also affected in the absence of this factor [16] and these events appear to hormone-independent. Interpretation of hormonal effects on gonocyte numbers in this period is complicated by the simultaneous increase in Sertoli cell number and it is possible that the increase in gonocyte numbers is, in part, a response to increased Sertoli cell numbers. Neonatal changes in Sertoli cell number are androgen-dependent but probably not FSH-dependent (although there are conflicting data) [11,18,19] which would suggest that effects of FSH on germ cell numbers in this period are not mediated through Sertoli cell proliferation. An earlier study has reported that gonocyte/spermatogonial numbers are unaffected by FSH suppression through passive immunisation during a similar period in the rat [20]. The reason for this discrepancy with the mouse data is not clear, it may indicate a species-dependent difference or it may be due to incomplete FSH suppression in the neonatal rats.

3. The first wave of spermatogenesis

Spermatogenesis starts soon after SSC differentiation and the first wave of spermatogenesis completes around day 40–45 in the rodent [21]. This first wave develops, therefore, in an environment which differs from subsequent waves in both the structure/activity of the testis and the hormonal environment. A further difference is a marked, BAX-regulated surge of apoptosis which occurs in the germ cells, particularly the pachytene spermatocytes, during the first wave [22,23]. This apoptotic event appears to be essential for subsequent waves of spermatogenesis, perhaps through synchronising the Sertoli cell/germ cell ratio [22]. Reduction in FSH levels during this period causes a marked increase in germ cell death suggesting that FSH normally acts to limit the apoptotic wave [20,24,25]. It has been reported that systemic injection of testosterone also inhibits apoptosis [22] suggesting a role for androgens in this process but it is not clear from this study whether intratesticular testosterone levels or circulating FSH were affected. Other evidence, however, also suggests that androgens will reduce spermatocyte apoptosis in the pre-pubertal rat consistent with a role for androgens in limiting apoptosis during the first wave [26]. Recent studies using FSHRKO and SCARKO mice have shown that there is a significant decrease in germ cell numbers in both groups at 20 days of age, during the first

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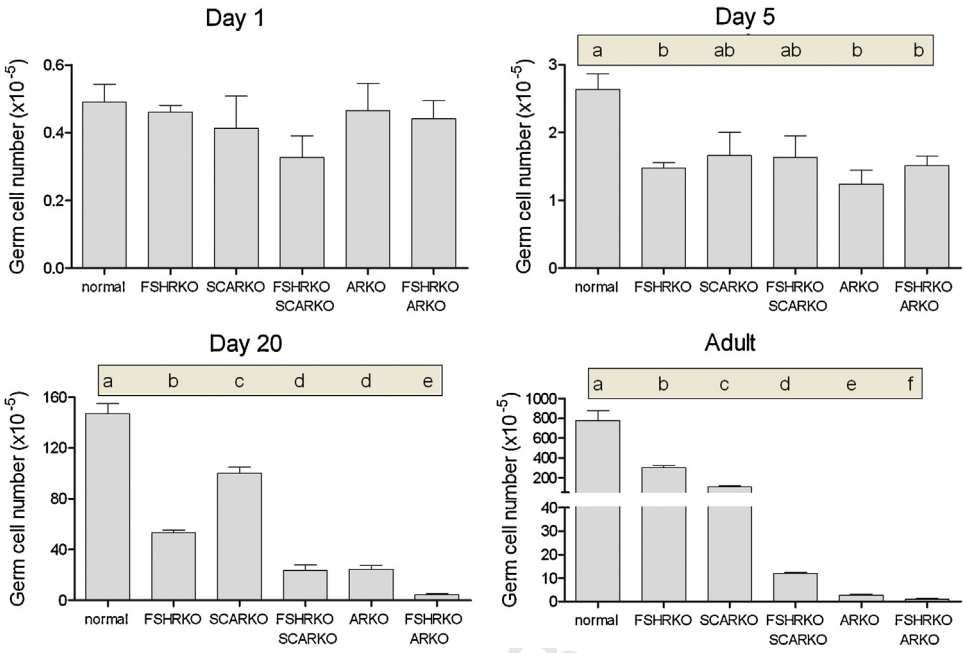

Fig. 1. Role of FSHR and AR in determining germ cell number during development. Total germ cell number per testis was measured in normal, FSHRKO, SCARKO, FSHRKO.SCARKO, ARKO and FSHRKO.ARKO mice during post-natal development. The data shows that gonocyte numbers are unaffected by loss of hormone action on day 1 but by day 5, when spermatogonia are present there is a similar loss of germ cells in most groups. By day 20, when spermatocytes are present and shortly before they enter meiosis, loss of FSHR (FSHRKO) was more marked than loss of AR in the Sertoli cells (SCARKO). Total loss of AR (ARKO) had the most marked effect of the single knockouts. Double knockouts (FSHRKO.SCARKO and FSHRKO.ARKO) had a greater effect than either knockout alone. A more detailed analysis of adult data is shown in Fig. 4. Results show the mean ± SEM of 3–6 animals per group at each individual age.
Reproduced from [4] and see this reference for analysis details.

spermatogenic wave but before completion of meiosis [4] (Fig. 1). This is also consistent with the hypothesis that both hormones act to limit apoptosis although reduced germ cell proliferation is also likely to contribute to the effects seen. What is interesting from this data, however, is that there is a more marked loss of germ cells in the FSHRKO mouse than in the SCARKO mouse while the greatest loss (not including double knockouts) was in the ARKO mouse (Fig. 1) This data shows that androgen action is critical at this age but also that the effects are largely mediated through cells other than the Sertoli cells–the most likely candidates at this age being the PTMC [6]. With respect to Sertoli cell-dependent hormonal regulation of germ cell development, this appears to be most sensitive to FSH in the prepubertal period. It is also worth noting that even before germ cells complete meiosis there is an additive and, possibly, synergistic effect of both hormones (seen in the double knockout FSHRKO.SCARKO and FSHRKO.ARKO mice) [4]. The first wave of spermatogenesis is somewhat unusual, therefore, compared to subsequent waves. It has been suggested that the apoptotic surge may be critical to ensure normal spermatogenesis in the adult and this may be a contributory cause of the sub-maximal fertility seen in the adult FSHRKO mouse [27].

4. Spermatogenesis in the adult

4.1. Follicle-stimulating hormone

Until the mid 1990s the general consensus was that FSH is essential for the initiation of spermatogenesis and that in the adult it is required to maintain normal quantitative germ cell production [28,29]. It was contrary to expectations at the time, therefore, when transgenic mice lacking FSH or its receptor (FSHR) were shown to be fertile, albeit with a reduced germ cell number [30–34]. Closer study of FSHRKO and FSHβKO mice showed a reduction in numbers of spermatogonia, spermatocytes and spermatids [30,34] from which it could be concluded that FSH acts to increase the number of spermatogonia and the entry of these cells into meiosis. It was less clear whether FSH would also stimulate completion of meiosis (ie formation of round spermatids) but comparison with SCARKO mice showed that even in the presence of normal FSH levels round spermatids would not form in the absence of AR on the Sertoli cells [30,35]. Previous studies had shown that injection of *hpg* mice with FSH or transgenic expression of FSH in these animals would increase numbers of spermatogonia and spermatocytes and induce round spermatid formation [36–39]. This effect on spermatid formation was, however, subsequently shown to be due to stimulation of testicular androgen levels since no effect of FSH is seen in *hpg*.SCARKO or *hpg*.ARKO mice [36]. This also serves to illustrate another problem when studying hormonal control of spermatogenesis; the hormones that we now have available do not suffer from cross contamination, as they once did, but it can be difficult to isolate the effects of a single hormone as they often affect levels of other hormones which act on the testis (an interesting example being stimulation of pituitary FSH secretion by estrogen in the *hpg* male [40]).

While FSHRKO and FSHβKO mice show what happens to adult spermatogenesis when there is lack of FSH action during development we cannot be sure that they show the normal adult role of FSH in maintaining this process. As discussed above, this is because the hormone or its receptor are missing from the start of reproductive development and it is not clear how much the adult phenotype reflects abnormal development of spermatogenesis or abnormal maintenance of the adult state. Lack of FSH, for example, during the high apoptotic period of the first spermatogenic wave probably increases the overall number of cells dying which may have a knock-on effect on adult spermatogenesis [22]. As yet we are unable to study the effects of selective FSH ablation in the adult using transgenic models since no floxed *Fshr* animals are available. Previous studies have achieved selective reductions in FSH levels through passive immunisation or gonadotrophin ablation (using hypophysectomy, GnRH agonists or enhanced steroid negative feedback)

with or without androgen replacement. In hypophysectomised rats treated with androgens, for example, sperm production is significantly reduced, presumably because of loss of FSH [41,42], while FSH immunoneutralisation significantly reduces germ cell numbers with a clear effect on spermatogonial survival [43]. Similarly, in hypophysectomised or GnRH-immunised adult rats FSH-treatment acts to increase spermatogonial and spermatocyte number but has a limited and incomplete effect on spermatogenesis [44,45]. While these results generally agree with the data from FSHRKO mice there are, however, significant differences. In particular, it was postulated in two of these studies that FSH acts to promote the completion of meiosis in rats [41,44], an effect not seen in the transgenic mouse models (discussed above). This may be a species-dependent difference or may be related to issues with the particular models used and, certainly, most studies do not see any clear effect of FSH on formation of post-meiotic cells in the rat. In addition to pre-meiotic effects, it has been suggested that FSH also plays a role in the normal completion of spermiogenesis. In FSHRKO mice, study of ejaculated sperm shows alterations in DNA condensation and an increase in morphological abnormalities [46,47]. Interpretation of these studies, however, is complicated by the fact that testosterone levels are reduced in FSHRKO mice [48]. In addition, the ratio of round spermatids to mature sperm in the FSHRKO mouse is similar to control [30,46] which suggests that FSH is not involved in the process of spermiogenesis. Similarly, in FSH-treated GnRH-immunised rats there is little or no progression from round to elongated spermatids [44] and no effect of FSH is seen in the *hpg* mouse [39]. There is evidence that FSH may be involved in the process of spermiation (release of mature spermatids from the Sertoli cells) [49] but androgens also appear to be the more important regulators of this process [50].

The mechanism of FSH action on spermatogenesis is uncertain but FSH can alter rates of germ cell apoptosis [25,51] and there is also evidence that FSH can act to increase spermatogonial differentiation/proliferation [52–54]. A number of studies have shown that glial cell line-derived neurotrophic factor (GDNF) and fibroblast growth factor 2 (FGF2) are important for SSC self-renewal and survival [55,56] and it has been reported that FSH stimulates GDNF and FGF2 levels in Sertoli cells [57–60]. This suggests that one effect of FSH may be at the SSC niche although in a study designed to look at the effects of FSH on testicular function in the *hpg* mouse we did not see any change in *Gdnf* or *Fgf2* levels [61] and effects of FSH at the niche remain to be confirmed. A further role for FSH in the testis may be maintenance of Sertoli cell water balance as the cells show accumulations of fluid in FSHRKO mice [62]. This alters cell morphology and interactions between the germ cells and Sertoli cells and might be expected to reduce normal spermatogenic efficiency.

4.2. Androgen

The essential role that androgen plays in development and maintenance of spermatogenesis has been known since the 1930s [63] and has been emphasised by a recent paper which shows that precocious expression of ARs in Sertoli cells leads to premature spermatogenic development [64]. The role of androgens can also be clearly seen in any animal model in which androgen levels are severely reduced through hypophysectomy, GnRH-treatment, EDS-treatment or in gonadotrophin-deficient mice. In each case there is massive loss of pachytene spermatocytes and round spermatids, particularly at stages VII and VIII of the cycle, which can be reversed by treatment with testosterone [39,65–74]. Similarly, in mice lacking functional androgen receptors (*tfm* or ARKO) there is a significant loss of spermatocytes and failure of these cells to complete meiosis and form round spermatids [4,8,18,35]. The major effects of androgen on meiosis appear to be ensuring survival of pachytene spermatocytes, particularly in the mid-spermatogenic stages, and enabling diplotene spermatocytes to enter into meiotic division [30,35,39]. As with study of FSH action on spermatogenesis, however, some of the effects of AR ablation in the adult may be related to changes in the first wave of spermatogenesis and there is the added complication that *tfm* and ARKO mice are cryptorchid which, by itself, will markedly affect spermatogenesis. One transgenic approach that can be used to address both of these questions is that taken by Willems et al. [10] who developed an inducible ARKO model. Induction of AR ablation in this iARKO model is through the action of tamoxifen which, unfortunately, has the potential drawback that, by itself, it can cause marked disruptive effects on spermatogenesis, albeit at high doses [75]. In the study by Willems et al. [10] there were significant endocrine changes and a reduction in testis weight associated with the actions of tamoxifen [10] but the effects of tamoxifen on spermatogenesis in control animals did not appear marked while there was clearly a severe loss of spermatocytes and spermatids in iARKO mice (though not explicitly reported in this study) [10]. Overall, therefore, both transgenic and non-transgenic animal models show that the major effects of androgens are to maintain the spermatocytes and to allow these cells to complete meiosis.

Other effects of androgens on germ cell development are more complex or more difficult to study. For example, the role that androgens play in spermiogenesis and spermiation is not clear from most genetically-modified models of AR ablation since the germ cells do not reach the spermatid stage. The process can be studies in androgen-withdrawal models, however, and results indicate that the adhesion between Sertoli cells and spermatids is androgen-dependent since androgen action is required to prevent the retention and phagocytosis of mature, elongated spermatids and the premature release of round spermatids [50,72,73,76,77]. The effects that androgen have on spermatogonial development are also complex. Hypophysectomy will reduce spermatogonial cell numbers [78,79] which is reversed by testosterone. It has also been shown that androgen (DHT) will induce an increase in spermatogonial numbers in *hpg* mice [74,80] but not in *hpg*.SCARKO or *hpg*.ARKO mice [74] (Fig. 2). A similar effect of FSH is also seen in both of these models, however [36,78], suggesting that both hormones can stimulate spermatogonial development (discussed further below). Currently, it remains entirely uncertain how androgen regulation occurs and there is no evidence at present of androgenic effects at the germ cell niche.

While it is clear that there is an essential role for androgens in stimulating spermatogenesis it has also been shown in an interesting series of studies that suppression of testicular testosterone levels stimulates recovery of spermatogenesis after irradiation/chemotherapy damage and germ cell transplantation [81,82]. Suppression of testosterone will also induce spermatogonial development in juvenile spermatogonial (*jsd*) mutant mice [83,84]. This effect of testosterone suppression on the *jsd* mice has been shown to be due to increased testicular temperature in these animals [85] while the effect on germ cell transplantation may be due to disruption of the blood-testis barrier allowing the stem cells to reach the Sertoli cell niche [86]. The mechanism underlying the effect of androgen suppression on the spermatogonial block induced by irradiation is, however, not clear at present and remains an important area for study in order to prevent sterility caused by cancer chemotherapy.

Androgen receptors are expressed in most cell types in the testis, the major exception being the germ cells themselves and it is clear that androgen action in the testis is only mediated through the somatic cell populations [87,88]. The Sertoli cells express AR soon after birth in rodents [89] and study of the SCARKO mouse shows that androgens act through the Sertoli cells to regulate spermatocyte number and progress of these cells through meiosis [30,35]. More surprisingly, ablation of AR on the PTMC also

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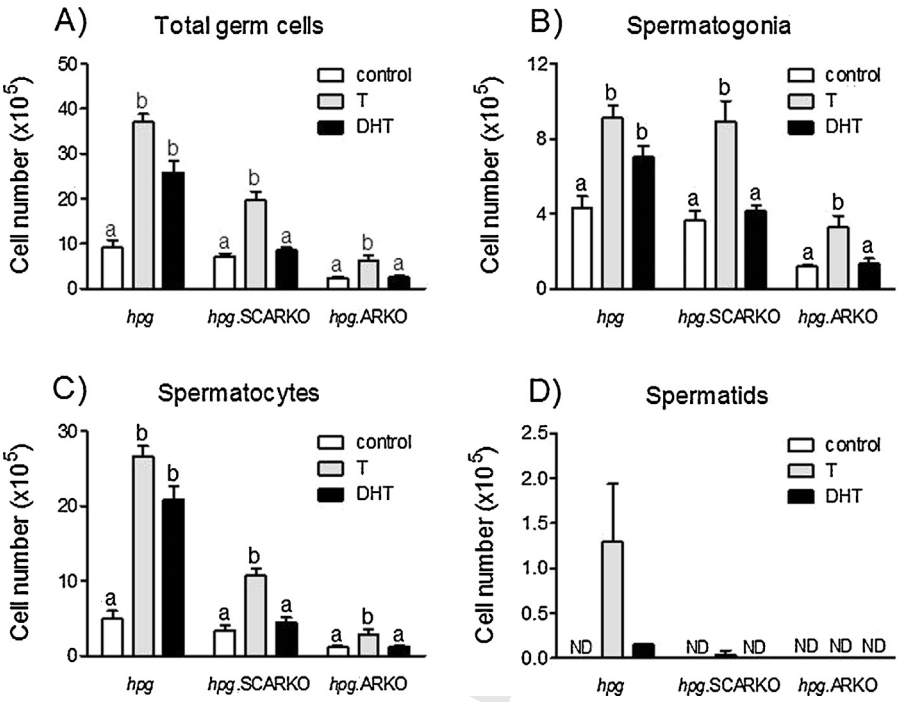

Fig. 2. Effect of androgen treatment on the number of total germ cells (A), spermatogonia (B), spermatocyte (C), and spermatids (D) in *hpg*, *hpg*.SCARKO, and *hpg*.ARKO mice. Adult mice were treated with T or DHT for 7d. Testosterone increased germ cell numbers in all three mouse groups but DHT (which cannot be aromatised to an estrogen) was only effective in the *hpg* group. This shows that androgens must act through AR on the Sertoli cells to stimulate spermatogenesis. The effects of T are largely due to increased FSH secretion in all three animal groups [74]. Results show the mean ± SEM. Within a particular animal type, groups with different letter superscripts were significantly ($P < 0.05$) different. ND, not detected.

Reproduced from [74].

has a major effect on spermatogenesis with a marked reduction in numbers of both spermatocytes and spermatids and a progressive loss of spermatogonia [6]. This demonstrates that androgens must also act through the PTMC to support spermatogenesis. A similar effect can be seen when FSHRKO.SCARKO mice are compared to FSHRKO.ARKO mice at 20 days of age, before normal testicular descent [4]. Total germ cell numbers in FSHRKO.ARKO mice are about 15% of those in FSHRKO.SCARKO mice, the main difference between the animals being that androgens can act through the PTMC in the FSHRKO.SCARKO animals but not the FSHRKO.ARKO mice (although an effect on Leydig cells cannot be ruled out). A similar, though less marked difference is also seen comparing the ARKO and SCARKO mice [4]. Comparison between *hpg* mice and *hpg*.SCARKO mice treated with androgen for 7 days shows, however, that androgen action through the PTMC alone does not stimulate spermatogenesis and that action through the Sertoli cells is essential (Figs. 2 and 3) [74]. Comparing a number of models (FSHRKO, hpg + A, Ptm-ARKO, Scarko, hpg.SCARKO + A [4,6,74]) it is clear that it is only when androgens are acting through *both* the Sertoli cells and the PTMC (i.e. normal, FSHRKO and *hpg* + A) that androgenic stimulation of spermatogenesis (increased numbers of spermatogonia, spermatocytes and post-meiotic cells) is seen.

Androgens probably act in diverse ways to stimulate spermatogenesis and a number of androgenic effects on the Sertoli cell are becoming clear although we currently have little idea what effect androgens have on the PTMC. Early studies using the AR-deficient *tfm* mouse reported that androgens are essential for formation of the blood-testis (Sertoli cell) barrier [90] and this has been shown, more recently, to be linked to altered expression of junctional proteins [91,92]. Interestingly, it has been reported that in SCARKO mice a barrier does form but formation is delayed and the barrier is defective [93]. The disruption caused to barrier formation would, however, be expected to alter the specific tubular environment required for spermatogenesis [93] and is a likely contributory factor to the loss of germ cell development in androgen-deficient animals. Androgens also act on the Sertoli cell to stimulate expression of the homeobox gene *Rhox5* [94] which may act as an intermediate transcription factor directing some of the actions of the AR [95]. Inactivating mutations in *Rhox5* have only a limited effect on spermatogenesis, however, leading to an increase in germ cell apoptosis and reduced sperm number and motility [96]. It is unlikely, therefore, that *Rhox5* is a major factor in androgen regulation of spermatogenesis. This gene is part of a large family of homeobox genes, however, and several are expressed in the Sertoli cell so there may be functional redundancy which reduces the effects of inactivating *Rhox5*. Androgens have also been linked to retinoic acid metabolism/action in the testis [97,98] and since retinoic acid has been linked to control of meiosis [99] this may be another route by which androgens control spermatogenesis. Most recently, a comprehensive list of androgen-regulated transcripts in mouse Sertoli cells has been identified using RNAseq and the RiboTag mouse [100]. In total, 938 mRNA transcripts were identified which are androgen-dependent and predominantly expressed in the Sertoli cells and this valuable new resource will undoubtedly help in identifying the mechanisms by which androgens regulate spermatogenesis. Interestingly, it has also been shown that, in addition to the normal pathway, testosterone can act through a non-classical pathway which involves AR recruitment of Src kinase and activation of the epidermal growth factor receptor [101,102]. Co-culture studies show that androgens act through this pathway to increase adhesion of spermatocytes and spermatids to Sertoli cells followed by release of elongated spermatids and mature spermatozoa [103]. For more information on this pathway see xxxxxxxx. Overall, since androgens have to act on both PTMC and Sertoli cells to regulate spermatogenesis, and given the variety of genes shown to be androgen-dependent, it is likely that a number of different pathways are involved in androgen regulation of spermatogenesis. Finally, androgens are also essential for Sertoli cell proliferation Q2

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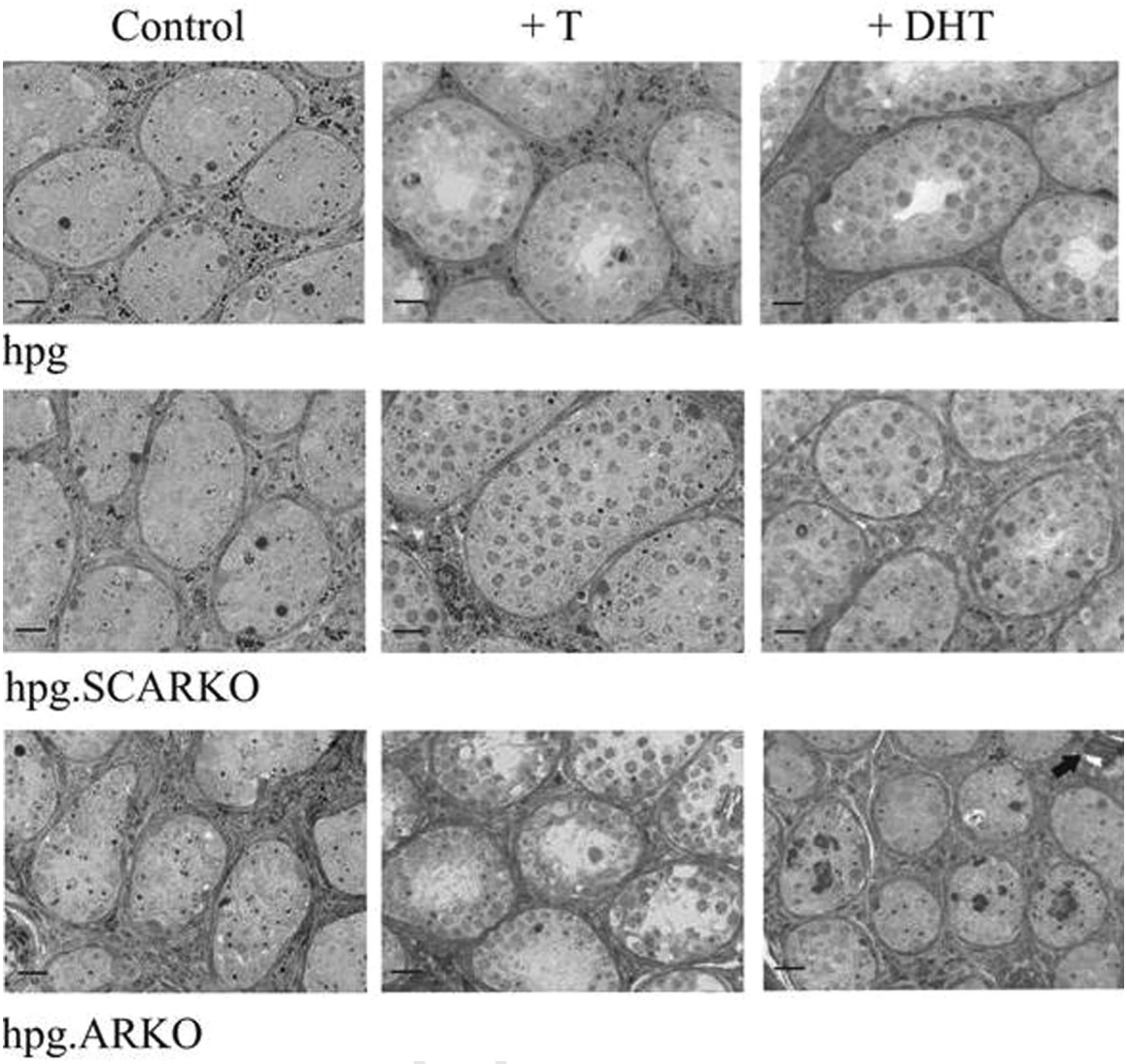

Fig. 3. Semithin sections showing the effect of androgen on testicular morphology in *hpg*, *hpg*.SCARKO, and *hpg*.ARKO mice. In untreated animals, spermatogenesis was severely disrupted with only spermatogonia and some spermatocytes present. Treatment with T increased germ cell numbers in all mice, although the effect was most marked in the *hpg* group. Treatment with DHT increased germ cell number only in *hpg* mice. In the hpg.ARKO group the black arrow indicates the presence of a microlith [146]. Bar = 20 μm.
Reproduced from [74].

during fetal and neonatal development [4,18,104] and, as Sertoli cell number regulates germ cell number [7], androgen stimulation during pre-pubertal development will also determine final germ cell numbers in the adult.

4.3. Estrogen

There is no doubt that estrogens can affect spermatogenesis but the effects are complex and the normal physiological role in the adult remains unclear. There is, however, good evidence to suggest that estrogen action is required in the neonate to ensure normal adult spermatogenesis. The complexity of estrogen action comes largely from the multiple indirect effects that the hormone has on the testis through endocrine regulation and through other tissues. Estrogens will stimulate spermatogenesis, for example, in the adult *hpg* mouse [105] but this effect is due to stimulation of FSH release from the pituitary [40]. On the other hand, exogenous estrogens will inhibit spermatogenesis in normal adult animals through inhibition of LH secretion and intratesticular testosterone levels [106]. Nevertheless, the testis has aromatase activity which converts androgens to estrogens and the testes of some species such as the horse produce large amounts of estrogens [107,108]. Several cell types in the testis, including germ cells, also express nuclear estrogen receptors (ERα and ERβ) as well as the membrane receptor GPR30 [109,110]. More pertinently, a physiological role for estrogens is suggested by the developing infertility in mice lacking aromatase (ArKO mice). Young ArKO mice are initially fertile but spermatogenesis degenerates with time with an arrest at early spermiogenesis and the appearance of multinucleated cells in the tubular lumen [111]. αERKO mice are also infertile and this appears to be through both estrogen-dependent and estrogen-independent mechanisms [112]. Estrogen-independent ERα signalling is required for concentrating epididymal sperm through regulation of fluid absorption by the epididymis [112,113]. Interestingly, estrogen-dependent ERα signalling is also required during the neonatal period to ensure normal adult spermatogenesis and fertility [112]. This effect is probably related to Sertoli cell maturation during this period although estrogens can increase spermatogonial numbers in neonatal rats [114]. ERβKO male mice are sterile but this does not appear to be due to defects in spermatogenesis [115] and GPR30KO

mice are reported to be normally fertile [116]. With respect to estrogen action on spermatogenesis, the clearest effects are seen on spermiogenesis and it has been suggested that acrosome biogenesis may be an estrogen-dependent process [117] which would be consistent with the developing spermatogenic failure seen in ArKO mice. Interestingly, estrogens will also stimulate spermatogonial differentiation following irradiation damage to the testis [118], an effect that is not related to testosterone suppression [119]. Thus, there is compelling evidence that estrogen plays a role in the early development of spermatogenesis (probably through the Sertoli cells) and it is clear that estrogens will affect spermatogenesis but the normal role that estrogens play in this process in the adult and the mechanisms of action remain to be determined.

4.4. Activin

Activins and activin-related proteins are produced by most testicular cell types [120] and probably should be described as local growth factors rather than hormones with respect to spermatogenesis. They do also act as hormones, however, and as such are included in this review (the same arguments could of course be made concerning androgens). Activin receptors have been identified in Sertoli cells and in germ cells with some receptors showing a stage-dependent pattern of expression [120]. Activin has been shown to have effects on SSC, spermatogonia and spermatocytes in culture [121–123] but the best evidence for a normal role in spermatogenesis comes from transgenic models. These can be complex to analyse since changes in activin/inhibin levels will also affect circulating FSH levels but in double knockout mice lacking FSH and the activin recptor type 2A (ACVR2A) there is a greater loss of epididymal sperm numbers and spermatogonia than in animals lacking FSH or ACVR2A alone [124]. There also appears to be a requirement for a normal expression pattern of activin secretion since animals with overexpression of the βA-subunit show progressive failure of spermatogenesis [125]. The activin-related proteins follistatin (FST) and follistatin-like 3 (FSTL3) are activin-binding proteins which act as antagonists to activin activity. Over-expression of FST, which would be expected to reduce local activin levels, causes infertility without clear effects on FSH levels [126]. Conversely, in mice lacking FSTL3 there is a clear increase in germ cell numbers although this is probably related mostly to a significant increase in Sertoli cell numbers [127]. Overall, available evidence suggests that activins may play a regulatory role in maintaining spermatogenesis and ensuring normal Sertoli cell development and activity.

4.5. Combined hormone action

While both androgen and FSH will act alone to stimulate spermatogenesis, it is clear that both hormones acting together are essential for full spermatogenesis. The combined effects of both hormones can be seen clearly in FSHRKO, SCARKO and combined FSHRKO.SCARKO mice [30] (Figs. 4 and 5). The FSHRKO.SCARKO mouse provides a baseline model with no direct endocrine stimulation of the Sertoli cells and in these animals there is a marked failure of spermatocyte development and absence of any cells completing meiosis [30]. The SCARKO mouse shows the effect of FSH on this model which is to stimulate spermatogonial and spermatocyte numbers while the FSHRKO mouse shows that androgens act to stimulate completion of meiosis [30]. It is only, however, when both hormones are present in the control animal that full, normal spermatogenesis occurs (Figs. 4 and 5). While it is clear that androgens are the main stimulatory factor regulating spermatogenesis, the two hormones act additively to stimulate entry of the germ cells into meiosis and synergistic to enhance completion of meiosis and entry into spermiogenesis [30]. Similar synergistic effects of FSH and testosterone are also seen in *hpg* mice with a marked

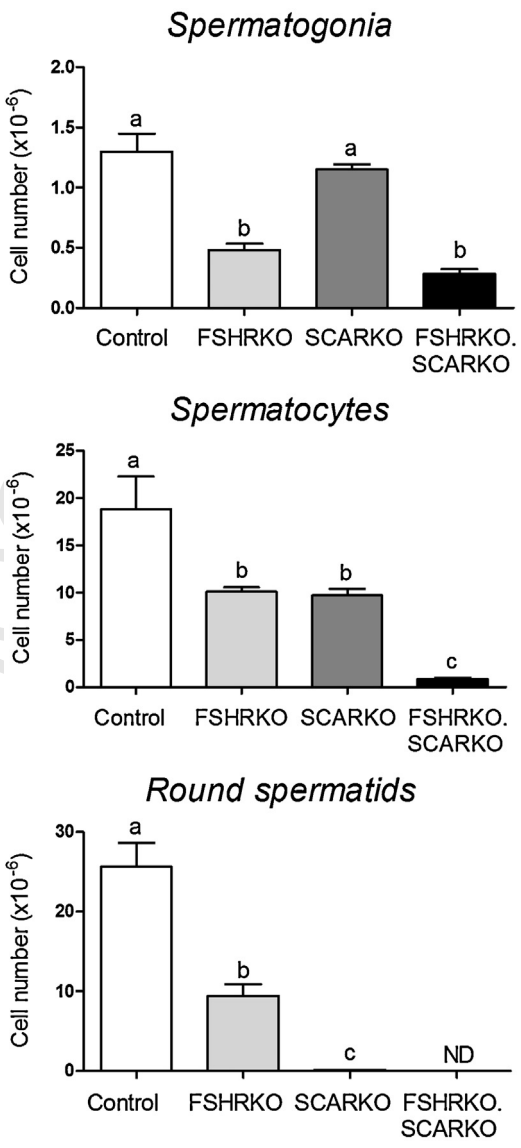

Fig. 4. Morphometric analysis of germ cell types in 8-wk-old testes from control, FSHRKO, SCARKO, and FSHRKO.SCARKO mice. The results show that in the absence of FSH or androgen action through the Sertoli cells (FSHRKO.SCARKO mice) tubules contain largely spermatogonia (see also Fig. 5). In the presence of FSH (SCARKO mice) there is a significant increase in spermatogonia and spermatocytes while in the presence of androgen (FSHRKO mice) there is an increase in spermatocytes and round spermatids. It is only in the presence of both hormones (control), however, that full spermatogenesis is seen. The mean ± SEM of four animals per group is shown. Groups with different letter superscripts are significantly different. Where there was a significant interaction between the effects of the two gene knockouts, this is indicated on the figure.
Adapted from [30].

increase in spermatid numbers in the presence of both hormones [39]. It has also been reported that in a rat model of suppressed androgen and/or FSH both hormones act synergistically to support spermiation [49] with a similar effect seen in the human [128].

Some of the additive effects of FSH and androgen are likely to be through the action of each hormone on different parts of the spermatogenic cycle but the mechanisms by which they act synergistically to increase completion of meiosis are not known. A number of potential synergistic mechanisms have, however, been previously described. For example, FSH has been shown to augment the action of testosterone and help induce tight junction formation at the Sertoli cell barrier [129]. Similarly, it has been reported that androgen may act to regulate FSH action on the Sertoli

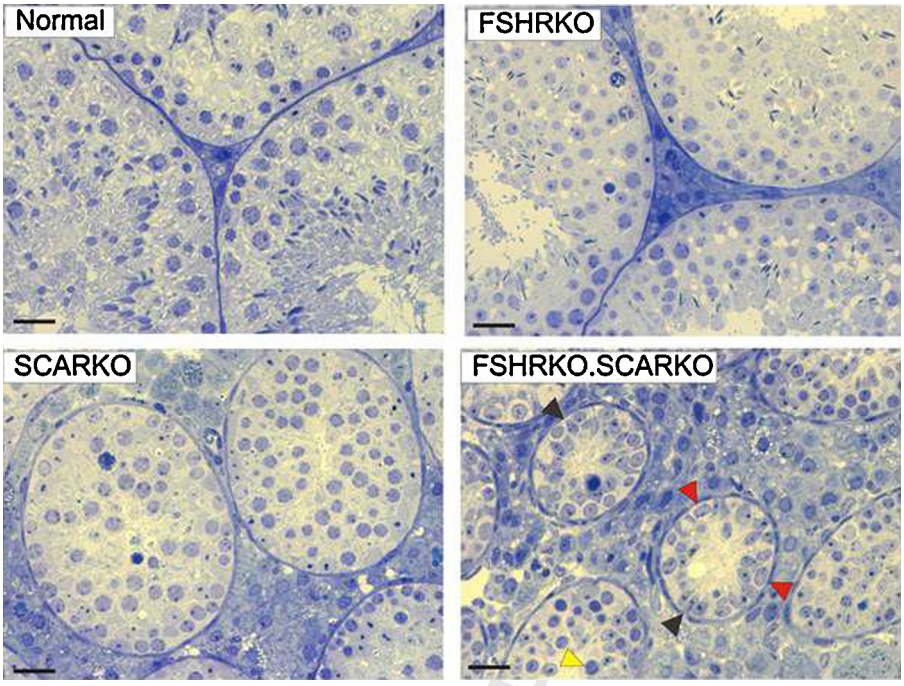

Fig. 5. Semithin sections from testes of 8-wk-old normal, FSHRKO, SCARKO, and FSHRKO-SCARKO mice. The FSHRKO mice contained all stages of spermatogenesis, although germ cell number was reduced. In SCARKOmice, spermatogenesis progressed through meiosis, but there was progressive loss of pachytene spermatocytes, and few secondary spermatocytes or round spermatids were observed. In FSHRKO.SCARKOmice, the tubules were of a smaller diameter with large numbers of Sertoli cells (black arrowheads) and smaller numbers of spermatogonia (red arrowheads). Spermatogonia entered meiosis, but development stopped at early pachytene in most cells (yellow arrowhead). Bar = 20 μm.

From Reference [30].

cell through the chromatin modifier metastasis-associated protein 2 (MTA2) [130]. In contrast, FSH has been shown to antagonise the effects of testosterone on germ cell attachment through the non-classical pathway [103]. Understanding the synergistic mechanisms underlying the action of these two hormones is not likely to occur, however, until we know how hormones act to regulate completion of meiosis.

5. Hormonal regulation of spermatogenesis in primates

Consistent with rodent models, spermatogenesis in primates is dependent upon FSH and androgen. Indeed, the effects of hypophysectomy on primates, including man, is more marked than in rodents with only Sertoli cells and spermatogonia left in the involuted tubules [131–133]. The phenotype is also more marked than that seen in the FSHRKO.SCARKO mouse [30] and suggests that both spermatogonial proliferation and onset of meiosis are more hormonally sensitive in primates. As with rodents, hypophysectomy and replacement studies show that androgens are essential for fertility in primates [131,134,135]. This is also demonstrated by the arrested spermatogenesis seen in individuals with mutations in LHβ which prevent secretion or receptor binding of the hormone despite normal testis descent [136,137]. In monkeys, androgens appear to be required, particularly, for conversion of type A pale (Ap) spermatogonia to type B, completion of meiosis, spermiogenesis and for spermiation [138]. The role of FSH in normal spermatogenesis in primates may be greater than in rodents but there remains some uncertainty. It has been shown in primates that unilateral orchiectomy will increase testis size and spermatogenesis in the remaining testis and this effect is associated with a long-term increase in circulating FSH levels [139,140]. Similarly, FSH will increase germ cell production in normal adult monkeys and humans [52,141], mainly through an increase in Ap spermatogonia entering the spermatogenic cycle. Finally, it has also been reported that FSH is as effective as LH in maintaining spermatogenesis in normal men with gonadotrophin suppression although it is not clear whether residual androgen levels in these men may have contributed to a synergistic effect with FSH [128]. While FSH will clearly stimulate spermatogenesis in primates, studies on the effects of inactivating mutations in the human are equivocal about the pivotal importance of this hormone during maintenance of normal spermatogenesis. Three separate studies have reported that inactivating mutations in FSHβ will cause azoospermia [142–144] suggesting FSH is critical for germ cell development but a reported inactivating mutation in FSHR was associated with oligozoospermia [145]. Results from a detailed stereological analysis of the effects of gonadotrophins in men suggest that FSH acts primarily to maintain spermatocyte numbers while both FSH and androgen maintain spermatid numbers [128].

6. Conclusions and future directions

The fundamental roles played by FSH and androgen in control of spermatogenesis were described over 70 years ago and further study in the intervening period has served to re-enforce the results of these early studies and to refine our knowledge of the action of these hormones. Overall, the primary effects of FSH and androgen appear to be similar in rodents, primates and other mammals (FSH acts to stimulate spermatogonial proliferation and entry into meiosis and T acts to ensure completion of meiosis and spermiogenesis). Androgens appear to be critical for spermatogenesis in all species and, while the relative importance of FSH may vary, it is clear that optimal spermatogenesis requires the action of both hormones. We are beginning to understand how FSH and androgen act to regulate germ cell development at the cellular and molecular level but this remains an area in which substantial advances are required. Current transgenic models are excellent for study of early germ cell development and the first wave of spermatogenesis but further work

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on the role of FSH and androgen in the adult will require development of inducible cell-specific knockouts, preferably not requiring use of tamoxifen. The critical role that the Sertoli cell plays in spermatogenesis has been, and remains, central to our understanding of spermatogenesis and hormone action. The breakthrough studies showing that androgens must also act through the PTMCs [6] illustrates, however, that the cellular regulation of spermatogenesis is more complex than previously anticipated and this must be a priority area for future study. Finally, the role that FSH plays in human spermatogenesis remains an interesting and important topic of study and further advances, probably through identification of more individuals with inactivating mutations, are awaited with interest.

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