Spermatogenesis is regulated by the 2 pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). This process is considered impossible without the absolute requirement of LH-stimulated testicular testosterone (T) production. The role of FSH remains unclear because men and mice with inactivating FSH receptor (FSHR) mutations are fertile. We revisited the role of FSH in spermatogenesis using transgenic mice expressing a constitutively strongly active FSHR mutant in a LH receptor–null (LHR-null) background. The mutant FSHR reversed the azoospermia and partially restored fertility of \( Lhr^{-/-} \) mice. The finding was initially ascribed to the residual Leydig cell T production. However, when T action was completely blocked with the potent antiandrogen flutamide, spermatogenesis persisted. Hence, completely T-independent spermatogenesis is possible through strong FSHR activation, and the dogma of T being a sine qua non for spermatogenesis may need modification. The mechanism for the finding appeared to be that FSHR activation maintained the expression of Sertoli cell genes considered androgen dependent. The translational message of our findings is the possibility of developing a new strategy of high-dose FSH treatment for spermatogenic failure. Our findings also provide an explanation of molecular pathogenesis for Pasqualini syndrome (fertile eunuchs; LH/T deficiency with persistent spermatogenesis) and explain how the hormonal regulation of spermatogenesis has shifted from FSH to T dominance during evolution.
Constitutively active follicle-stimulating hormone receptor enables androgen-independent spermatogenesis

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Introduction

It is textbook knowledge that spermatogenesis is impossible without luteinizing hormone–stimulated (LH-stimulated) testicular testosterone (T) production (1, 2). Human mutations and genetically modified mice provide further proof for this, as men harboring inactivating LHB and LHCGR mutations are hypogonadal and azoospermic, with knockout mice for the same genes exhibiting a similar phenotype (2). Surprisingly, the role of follicle-stimulating hormone (FSH), the other endocrine stimulus of spermatogenesis, has remained elusive. Although a long-held notion ascribes the hormone (FSH), the other endocrine stimulus of spermatogenesis, has distinct and irreplaceable effects.

transcription factor, it is justified to hypothesize that each hormone action, FSH acting through a GPCR with cAMP as second messenger and T activating the androgen receptor (AR), a nuclear transcription factor, is justified to hypothesize that each hormone has distinct and irreplaceable effects.

To delineate the cryptic role of FSH in spermatogenesis, we used the transgenic mouse model expressing a constitutively strongly activating Fshr point mutation (Fshr-CAM; D580H) in Sertoli cells (SC) under the human anti-Müllerian hormone promoter (8). These mice were crossed with female heterozygous Lhr+/− mice (9) with the expectation that the role of FSH could in this way be amplified and studied in isolation from the LH/T effects. The unexpected findings during the experiments challenge the dogma of T dependence of spermatogenesis because missing T action was completely compensated for by strong FSH stimulation. Our findings therefore herald potentially novel strategies into the treatment of human spermatogenic failure and suggest mechanisms for...
some unexplained aberrations of human spermatogenesis and the evolution of its hormonal regulation.

Results and Discussion

Our transgenic mouse model expressed a constitutively strongly activating (cAMP response >10-fold above basal) Fshr point mutation (Fshr-CAM; D580H) under the human anti-Müllerian hormone promoter (8), providing strict SC-specific expression of the transgene (10) and almost a 20-fold Fshr expression at mRNA level in comparison with WT mice. Unlike females with a robust ovarian and reproductive phenotype (8), the male littermates had no apparent abnormalities. Their testicular architecture (Figure 1B) and hormonal parameters (Supplemental Table 1; supplemental material available online with this article; https://doi.org/10.1172/JCI96794DS1) were as in WT (Figure 1A and Supplemental Table 1). Immunohistochemical localization of the FSH protein and RNAscope in situ hybridization of Fshr mRNA were confined to SC (Supplemental Figure 1), indicating that functionally meaningful leakage of the Fshr-CAM transgene to Leydig cells (LC) is highly unlikely. In the absence of any phenotype of the Fshr-CAM males, it is apparent that physiological FSH concentrations provide maximal SC stimulation. This may explain why only 2 activating FSHR mutations, detected serendipitously, have been described. One was a hypophysectomized man with persistent spermatogenesis (11) and the other a man with normal spermatogenesis in the absence of circulating FSH (12). Furthermore, testicular function appears normal in men with pituitary adenomas secreting excessive FSH (13, 14). Hence, there may be no phenotypic effect of enhanced FSH action in otherwise healthy men.

Strong Fshr activation in the double-mutant Fshr-CAM/Lhr−/− mice surprisingly reversed the hypogonadism and infertility of the Lhr−/− mice, with development of a near-normal male phenotype with increased testicular size and spermatogenesis (Figure 1C), comparable to that in WT and Fshr-CAM littermates (Figure 1, A and B). These mice, however, presented with delayed puberty (Supplemental Figure 2A). The anogenital distance, another androgen-dependent developmental parameter, was similar in the Fshr-CAM/Lhr−/− mice and control littermates at weaning (P20), but at puberty and thereafter, it was approximately 10% and 40% shorter in the Fshr-CAM/ Lhr−/− and Lhr−/− mice, respectively (Supplemental Figure 2B). Mating tests showed similar mounting behavior in Fshr-CAM/Lhr−/− mice and WT littermates, with evidence for copulatory plugs in females. However, the Fshr-CAM/Lhr−/− breeding pairs had lower frequencies of pregnancies and smaller litter sizes (Supplemental Table 2), with no evidence for embryonic lethality in offspring.

LH concentrations were markedly (10-fold) and FSH marginally (1.5-fold) elevated in the mice with Lhr−/− genotypes (Figure 2, A and B). Serum T in the Lhr−/− mice was very low (<0.01 nmol/l), while in Fshr-CAM/Lhr−/− mice, it recovered to about 40% of WT levels (Figure 2C). Similar trends, though with greater differences between genotypes, were observed in intratesticular T (iTT) concentrations (Figure 2D). The increased seminal vesicle (SV), epididymis, and testis weights (Supplemental Figure 2, C-E) and the observed testicular descent in Fshr-CAM/Lhr−/− mice demonstrated biological action of the partially recovered T production. However, the lack of LH suppression indicated that the recovered T production was insufficient to evoke negative feedback.

Sizes of the double-mutant mouse testes were indistinguishable from those of WT (Table 1 and Supplemental Figure 2E) and reflected on the appearance of fully developed seminiferous tubules, with typical stages of the seminiferous epithelial cycle.
Table 1. Testicular weights, seminiferous tubular diameters, and cell-type compositions

<table>
<thead>
<tr>
<th>Testis weight (mg)</th>
<th>WT</th>
<th>Fshr-CAM</th>
<th>Fshr-CAM/Lhr−/−</th>
<th>Lhr−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>105.3 ± 2.20</td>
<td>99.2 ± 4.90</td>
<td>90.8 ± 5.89</td>
<td>19.7 ± 0.86</td>
</tr>
<tr>
<td>Tubule diameter (μm)</td>
<td>199.7 ± 4.67</td>
<td>220.6 ± 8.02</td>
<td>207.7 ± 6.20</td>
<td>118.2 ± 2.68</td>
</tr>
<tr>
<td>SC (×10⁶/testis)</td>
<td>14.6 ± 0.24</td>
<td>12.4 ± 0.41</td>
<td>12.1 ± 0.51</td>
<td>4.3 ± 0.33</td>
</tr>
<tr>
<td>Spermatogonia A (×10⁶/testis)</td>
<td>8.3 ± 0.80</td>
<td>7.0 ± 0.55</td>
<td>7.8 ± 0.82</td>
<td>16.2 ± 1.91</td>
</tr>
<tr>
<td>Spermatogonia B (×10⁶/testis)</td>
<td>1.83 ± 0.22</td>
<td>2.1 ± 0.24</td>
<td>1.7 ± 0.18</td>
<td>2.61 ± 0.23</td>
</tr>
<tr>
<td>PS (×10⁶/testis)</td>
<td>69.4 ± 4.74</td>
<td>62.3 ± 2.87</td>
<td>60.4 ± 4.23</td>
<td>60.0 ± 5.15</td>
</tr>
<tr>
<td>RS (×10⁶/testis)</td>
<td>205.6 ± 8.14</td>
<td>179.4 ± 9.71</td>
<td>192.9 ± 4.91</td>
<td>9.8 ± 1.46</td>
</tr>
<tr>
<td>Elongated sperm (×10⁶/testis)</td>
<td>57.3 ± 3.55</td>
<td>49.7 ± 1.36</td>
<td>29.1 ± 2.76</td>
<td>0</td>
</tr>
<tr>
<td>Spermatogonia B/SC ratio</td>
<td>1.92 ± 0.16</td>
<td>2.25 ± 0.18</td>
<td>2.09 ± 0.23</td>
<td>10.40 ± 1.43</td>
</tr>
<tr>
<td>Spermatogonia B/RC ratio</td>
<td>1.26 ± 0.16</td>
<td>1.68 ± 0.20</td>
<td>1.42 ± 0.19</td>
<td>6.36 ± 0.72</td>
</tr>
<tr>
<td>RS/SC ratio</td>
<td>14.3 ± 0.48</td>
<td>14.5 ± 0.74</td>
<td>16.2 ± 0.80</td>
<td>2.3 ± 0.30</td>
</tr>
</tbody>
</table>

The number of observation is n = 5 mice/group (mean ± SEM). Groups with different superscript letters differ significantly from each other (P < 0.05; ANOVA/Newman-Keuls). Nondetectable results were assigned a value of 0 for statistical analysis.

and presence of elongated and mature spermatids (Figure 1C). A noticeable difference common to both Lhr−/− genotypes was the apparent lack of mature LC (Figure 1, C and D). Stereological assessment per testis (Table 1) and per mg testis (Supplemental Table 3) indicated approximately 15% less SC per testis of the Lhr−/− mice, compared to the WT (Figure 1, C and D). Stereological assessment after treatment (Supplemental Table 4) confirmed the efficacy of flutamide treatment. Previously, the same antiandrogen treatment in 12-month-old Lhr−/− mice completely blocked spermatogenesis at the RS stage; however, spermatogenesis was qualitatively complete in control Lhr−/− mice and driven by the residual low iTT level (2% of normal) (19). Surprisingly, identical antiandrogen treatment of Fshr-CAM/Lhr−/− mice brought about only shrinkage of the SVs, without affecting testicular size, cell-type composition, and sperm maturation (Figure 1F and Supplemental Table 4). Hence, upon complete blockage of androgen action, the constitutively active FSHR maintained spermatogenesis in these mice.

Previously, a study on the role of FSH in mouse spermatogenesis compared Lhr−/− and hypogonadal gonadotropin–deficient hpg mice, expressing through transgenesis either human FSH or a mildly constitutively active form of human FSHR (20). Both presented with normal to high-normal FSH action and low T levels, which, relative to hpg controls, led to increased SC numbers, enhanced spermatogonial proliferation, and some meiotic development, but no mature spermatids. FSH stimulation alone in these models was unable to evoke complete spermatogenesis without the critical involvement of LH-stimulated T production. In contrast, our findings demonstrate that stimulation of spermatogenesis with strong FSH effect alone is possible.

Quantification of selected androgen-dependent SC genes Drd4, Rhox 5, and Eppin (2) demonstrated their clearly decreased expression in the flutamide-treated WT testes in agreement with their low expression in the androgen-deprived Lhr−/− testes (Figure 3, D and E). In contrast, no reduction in the high expression of these androgen-dependent genes was found in flutamide-treated Fshr-CAM/Lhr−/− testes (Figure 3E), indicating that the strong Fshr-CAM signaling was able to maintain the expression of genes considered strictly androgen regulated. A similar expression pattern was found with the indirectly androgen-dependent postmeiotic germ cell-specific gene Aqp8 (21), apparently reflecting the persistence of testicular postmeiotic germ cells, normally present in the testis only through androgen action, but maintained by Fshr activation in the flutamide-treated Fshr-CAM/Lhr−/− testes. Another SC-specific gene, Gata-1, is resistant to androgen action, but downregulated by paracrine effects from postmeiotic germ cells (22). Hence, it was upregulated in the flutamide-treated WT testes, consequent to postmeiotic germ cell depletion, but not in the Fshr-CAM/Lhr−/− testis.
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sate for missing or insufficient T action, e.g., in oligozoospermia due to partial androgen resistance. Indeed, a study using 3- to 6-fold higher than the standard dose of FSH (75 IU, 2 to 3 times weekly) showed significant stimulation of spermatogenesis in idiopathic oligozoospermia (31). Besides increased FSH doses, the recently developed small molecule allosteric agonists of glycoprotein hormones (32) could offer a future alternative to boost FSHR activation. These findings may also explain the mechanism of persistent spermato genesis in a hypophysectomized male with activating FSHR mutation (11) and suggest a role for FSH in the LH/T-deficient Pasqualini syndrome (fertile eunuch) (33). Clearly, spermatogenesis is possible without T, and the potential of strong FSH stimulation in the treatment of spermatogenic failure needs further attention. Finally, our findings provide insight into the perplexing shift in the hormonal regulation of spermatogenesis during evolution from FSH in teleost fishes to LH/T dominance in mammals (34).

Methods

Statistics. Single comparisons were performed with unpaired 2-tailed Student’s t tests and multiple comparisons using ANOVA and Newman-Keuls post hoc test. All data sets are presented as mean ± SEM, unless otherwise stated. P < 0.05 was considered statistically significant.

Study approval. All procedures conformed to the Imperial College London Animal Welfare Protocol and were approved in accordance with the regulations and standards of the UK Home Office Animal Scientific Procedures Act (ASPA) 1986 and the European Union Directive (2010).
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For additional information, see Supplemental Methods.

Author contributions
OOO, HP, and ITH designed the study. OOO, HP, AP, LV, MC, MD, LS, LO, and BK performed experiments and collected data. OOO, HP, AP, LV, MC, MD, LS, NAR, and ITH analyzed the data. OOO and ITH drafted the manuscript, with final editing from all authors.

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