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Follicle-Stimulating Hormone Regulates Both Sertoli Cell and Spermatogonial Populations in the Adult Photoinhibited Djungarian Hamster Testis[1]

Sarah J. Meachem,[2,3,4] Peter G. Stanton,[3] and Stefan Schlatt[4,5]

Prince Henry's Institute of Medical Research,[3] Clayton, Victoria 3168, Australia
Institute of Reproductive Medicine,[4] University of Münster, 48129 Münster, Germany
University of Pittsburgh School of Medicine[5], Pittsburgh, Pennsylvania, 15261

ABSTRACT

The hormones that regulate spermatogonial development are ill defined, in part due to lack of appropriate experimental models. The photoinhibited hamster model provides a rich source of spermatogonia, thus making it an ideal model to study their control. This study aimed to assess the effects of FSH, in the absence of testosterone, on the reinitiation of Sertoli cell and spermatogonial development in the photosensitive adult Djungarian hamster. Hamsters raised under long photoperiods (LD, 16L:8D) were exposed to short photoperiods (SD, 8L:16D) for 11 wk, leading to suppression of gonadotropins and regression of testicular function. Groups of 10 animals then received FSH alone or in combination with the antiandrogen, flutamide, for 7 days. Two control groups maintained either under long or short photoperiods were treated with vehicle. Sertoli and germ cell number were then determined using the optical disector (*sic*) stereological technique. The number of Sertoli cells, type A spermatogonia, type B spermatogonia/preleptotene spermatocytes, and leptotene/zygotene spermatocytes were suppressed in SD controls to 66%, 34%, 19%, and 10% (all $P < 0.01$) of long-day control values, respectively. Later germ cell types were not detected. FSH treatment, with or without flutamide, increased Sertoli cell number ($P < 0.01$) to normal long-day values. Similarly, FSH treatment in the absence/presence of flutamide increased type A spermatogonia, type B spermatogonia/preleptotene spermatocytes, and leptotene/zygotene spermatocytes to ~85%, 69%, and 80% (all $P < 0.01$) of long-day controls, respectively. Our data demonstrate that the reinitiation of spermatogonial maturation in this model is dependent on FSH in the presence of an antiandrogen. Surprisingly, the adult Sertoli cell population in this model is also hormone dependent. This naturally occurring model provides a unique opportunity to understand the mechanisms (apoptotic and/or proliferative) by which FSH regulates Sertoli and germ cell development in the adult animal.

follicle-stimulating hormone, flutamide, germ cells, Sertoli cells, spermatogenesis, stereology, testis

INTRODUCTION

The hormones that regulate spermatogonial development are ill defined, in part due to a lack of appropriate experimental models and methods identifying spermatogonial subtypes that afford an appreciation of alterations in the size of a relatively small cell population. Germ cell transplantation as a functional assay has provided valuable insights into stem and differentiating spermatogonial differentiation and expansion [1–3]. It is apparent that the spermatogonial population is, at least in part, dependent on gonadotropins, as evidenced by an ~50% reduction in the spermatogonial population, as determined by modern stereological methods, following chronic withdrawal of pituitary gonadotropins (and, as a consequence, chronic withdrawal of testicular testosterone) by hypophysectomy [4] and GnRH immunization in rats [5, 6]. In monkeys [7, 8] and man [9, 10], a more profound involution of the spermatogonial population has been reported (65–90% reduction from normal) following gonadotropin withdrawal, suggesting hormonal dependencies of spermatogonia vary across species.

In the Djungarian hamster, photoinhibition suppresses pituitary LH (and, as a result, testicular testosterone) and FSH and, as a consequence, spermatogenesis is disrupted primarily during spermatogonial development [11–13]. As a result of the disappearance of later germ cell forms, these animals show a marked increase in the proportion of spermatogonia, making it an ideal model to study aspects of spermatogonial regulation in vivo. Few studies have used this model to study the endocrine control of spermatogenesis. Even so, sperm production [12] and fertility [14] in the photoinhibited hamster has been shown to be highly dependent on FSH, with testosterone only being reported to be necessary for mounting behavior [12]. Interestingly, spermatogenesis spontaneously resumes after lengthy (15-wk) periods of short-day conditions, with this resumption being associated with a prompt rise in serum FSH but not testosterone [13, 15].

The present study sought to determine the short term (7 days) effect of FSH, in the absence of androgens, on the reinitiation of spermatogonial development and later germ cells in the photoinhibited Djungarian hamster. To determine changes in cell populations, the optical disector [sic] stereological technique was used to quantify spermatogonial, spermatocyte, spermatid, and Sertoli cell numbers following hormonal manipulation. Serum and testicular hormone levels were monitored in all groups including pituitary LH- and FSH-β subunit mRNA expression. This study found that FSH regulates Sertoli cell and spermatogonial numbers with some support for spermatocyte maturation. This naturally occurring model provides a unique oppor-

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[2]Correspondence: Sarah Meachem, Prince Henry's Institute of Medical Research, Monash Medical Centre, Block E, Level 4, 246 Clayton Road, Clayton, Victoria, 3168, Australia. FAX: 61 3 9 594 6125;
e-mail: sarah.meachem@phimr.monash.edu.au

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TABLE 1. Primer-specific conditions used for PCR amplification of FSHβ and LHβ subunits.

Name and accession no.	Primer sequence	Product size (bp)	Primer conc. (pmol)	Mg^{2+} conc. (mM)	Annealing temp. (°C)	Extension time (sec)	Data temp. (°C)*	PCR product melt temp. (°C)†
Hamster FSHβ	Sense 5′-ACCCAGCCAGACCCAATAC-3′	140	40	3.0	58	12	82	86.3
AF106914	Antisense 5′-CAGTCAGTGCTGTCGCTGTC-3′							
Hamster LHβ	Sense 5′-GGCCTGTCAACGCAACCCT-3′	175	40	2.5	61	12	87	91.7
AF106915	Antisense 5′-GCGGACAGAAGCGAAGTGTA-3′							

* Refers to the temperature at which the fluorescence of the PCR product was quantified during analysis.
† PCR products have characteristic melting points (T_m); the T_M for a DNA product is defined as the temperature at which half the DNA helical structure is lost, and is determined by melting temperature analysis.

tunity to understand the mechanism by which FSH regulates aspects (proliferation and apoptosis) of the Sertoli and germ cell development in the adult animal.

MATERIALS AND METHODS

Animals

Fifty adult Djungarian hamsters (also called Siberian hamsters, *Phodopus sungorus*) were bred, raised, and housed for 150 ± 30 days under artificial long photoperiods (16L:8D) and constant temperature (22°C) with free access to pelleted food and water in the colony of the Institute of Reproductive Medicine, University Münster, Germany [for details, see 16]. All experiments were in accordance with local guidelines and with German law on the care and use of laboratory animals. All hamsters included in the experiments had large testes, as determined by palpation at the onset of the experiment.

Experimental Design

Suppression phase by photoinhibition. Photoinhibition of reproductive function was induced by transfer of 40 hamsters from long- to short-day photoperiods (8L:16D) for 12 wk. One group of hamsters (n = 10) remained under long photoperiods as reproductively active long-day controls. The response to photoinhibition was assessed by palpation, after which all hamsters with no palpable testes were included in the study. Hamsters were then allocated to one of five groups, three groups of which received hormone treatment.

Recovery phase by hormone treatment. Groups of animals received FSH reconstituted in sterile NaCl (0.154 M) (recombinant human [rh]-FSH; Gonal F; 6 IU/day s.c.; Serono, Sydney, Australia) alone (group 1) or in combination with the antiandrogen, flutamide (group 2) (10 mg/kg/day s.c.; Sigma, St Louis, MO), as described [17] and shown to be sufficient to block androgen action in the testes and accessory organs of adult rats, which was dissolved in a mixture of absolute ethanol and sesame oil. One group received flutamide alone (group 3). Two groups of control animals were used, photoinhibited (short day, group 4) and photostimulated (long day, group 5), both of which received sterile NaCl (0.154 M). All animals were killed by decapitation under anesthesia after 7 days of hormone treatment.

Tissue Collection

Trunk blood was collected and allowed to clot overnight at 4°C before serum collection for hormone assays. Testes, prostates, and pituitaries were then excised and weighed. Pituitaries and the right testis of each animal were snap frozen on dry ice and stored at −75°C for analysis, while the left testis was immersion-fixed in Bouins solution for less than 5 h. For testes >50 mg, tissue was sliced into 2- to 3-mm-thick slabs orthogonal to the long axis of the testis. For long-day hamster testes, two of the four slabs were selected using a systematic random-sampling scheme; for testes between 50 and 150 mg, one of the two slabs were selected; while <50 mg testes were processed whole and embedded into hydroxyethylmethacrylate resin (Technovit 7100; Kulzer and Co. GmBH, Friedrichsdorf, Germany) according to the manufacturer's instructions. Thick (25-μm) resin sections were serially cut (Supercut Microtome; Reichert Jung 2050, Nussloch, Germany), stained with the periodic acid-Schiff reaction reagents and counterstained with Mayer hematoxylin, as previously described [18] for the determination of cell number. All slides were masked before estimation of germ cell number.

Cell Number Estimates Using the Optical Disector Method

The optical disector method [19] was used to determine the total number of cells per testis, as previously described [20, 21]. Hamster germ cells were identified using the morphological criteria of Van Haaster and De Rooij [22], which resemble criteria used for rats [23] as previously described [24]. A total number of 80–160 nuclei of each cell type were counted per animal. A set of two unbiased counting frames in each field (area of each frame being 459 μm^2) were employed to count Sertoli cells and all germ cells types, except in long-day controls, where one unbiased counting frame in each field (2923 μm^2) was employed to count Sertoli and early germ cells (spermatogonia through to zygotene spermatocytes). Cells were counted in a depth of 10 μm. The final screen magnification was ×2708. As previously determined, no correction for shrinkage was required [5, 20].

The number of Sertoli and germ cell per testis were estimated for all groups. Germ cells were counted in the following categories: type A spermatogonia (across all stages)/intermediate spermatogonia (associated with stages I–IV), type B spermatogonia/preleptotene spermatocytes (associated with stages V–VIII), leptotene/zygotene spermatocytes (associated with stages IX–XII), pachytene spermatocytes (associated with stages I–XII), round (associated with stages I–VIII), and elongated spermatids (associated with stages I–XII).

Testosterone Assay

Serum and testicular testosterone levels were measured by RIA after ether extraction, as described previously [25]. Testicular cytosols were prepared by homogenizing the whole testis (<100 mg) in 250 μl or (>100 mg) in 1 ml of 0.01 M phosphate buffer, pH 7.4, 0.154 M NaCl for 30 sec with a tissue disperser (Ultra-Turrax; Janke and Kundel KG, Staufen, Germany). The homogenate was centrifuged at 12 000 × *g* for 15 min, and the supernatant collected and frozen at −75°C. Serum samples were assayed in duplicate across a single assay, as were cytosol samples. Assay sensitivity for serum and cytosols was 0.4 ng/ml and 0.3 ng/ml, respectively, and the within-assay coefficients of variation for both assay were 6%.

FSH Assay

Serum FSH was measured by a heterologous RIA (Amersham FSH-kit RPA 550; Amersham Biosciences, Piscataway, NJ) as previously described for rats [17] and hamsters [13]. National Institute of Diabetes and Digestive and Kidney Diseases FSH standards (rat FSH-RP-2), tracer (FSH-I-6), and antisera rFSH-11 were used. Quality controls were normal (values ranged between 13 and 18 ng/ml) and castrate (50–80 ng/ml) rat serum. Additional long- (n = 10) and short-day (n = 10) hamster serum samples were measured, with values between groups showing only modest differences, corresponding to values presented in Table 2. The detection limit of the assay was 3.2 ng/ml, and the within-assay coefficient of variation was 9.8%.

Extraction and Quantitation of Pituitary FSHβ and LHβ Subunit mRNA Levels

Total RNA was extracted from pituitaries using a total RNA extraction kit (Qiagen; Hildens, Germany) according to the manufacturer's instructions. Any contaminating DNA was removed using the DNase-free kit (Ambion, Austin, TX), and samples were stored at −80°C. Total RNA concentrations were determined using the Ribogreen fluorescence RNA assay (Molecular Probes, Eugene, OR) as described elsewhere [26]. Re-

TABLE 2. Body and organ weights and hormone concentrations (± SEM, n = 10/group) in the photostimulated (long day) adult Djungarian hamsters that were exposed to short photoperiods (short day) for 11 weeks.

Treatment*	Body weight (g)	Testis weight (mg)	Prostate weight (mg)	Serum FSH (ng/ml)	Serum testosterone (ng/ml)	Testicular testosterone (ng/testis)
Long day control	53.14 ± 2.31[a]	455.6 ± 33.7[a]	179 ± 20.5[a]	4.98 ± 0.60[a]	1.13 ± 0.12[a]	196 ± 20.70[a]
Short day control	41.73 ± 1.51[b]	27.2 ± 1.22[b]	28.5 ± 3.68[b]	3.64 ± 0.13[a]	0.86 ± 0.12[a]	9.92 ± 1.55[b]
+FSH	43.05 ± 1.49[b]	70.4 ± 2.18[c]	28.2 ± 2.42[b]	65.7 ± 10.5[b]	0.66 ± 0.06[a]	7.39 ± 0.92[b]
+FSH + Flut	41.21 ± 2.0[b]	75.7 ± 5.71[c]	43.0 ± 7.32[d]	51.4 ± 5.20[b]	1.02 ± 0.17[a]	20.0 ± 3.85[d]
+Flut	41.16 ± 1.60[b]	28.5 ± 3.17[b]	23.8 ± 3.63[b]	4.20 ± 0.26[b]	1.52 ± 0.24[a]	21.37 ± 5.08[d]

* Treatment consisted of FSH (6IU/day, sc) alone or with flutamide (Flut, 10mg/kg/day, sc) for 7 days.
[a–d] Different superscripts indicate significant differences between the treatment groups at or less than $P < 0.05$; detection limits of assays are: 3.2ng/ml FSH, 0.4ng/ml serum, and 0.3ng/ml testicular testosterone.

verse transcription was performed on 150 ng total RNA/sample using AMV-reverse transcriptase (8 U) (Roche, Mannheim, Germany), random hexamer primers (200 ng) (Amersham Biosciences), dNTPs (20 nmol each) (Roche), RNasin (20 U) (Promega, Madison, WI) and 5× reaction buffer (Roche) in a final volume of 20 μl for 90 min at 46°C, after which samples were heated for 2 min at 95°C before storage at −20°C.

Quantitative real-time polymerase chain reaction (PCR) analysis was performed using the Roche Lightcycler (Roche, Mannheim, Germany) and the FastStart DNA Master SYBR-Green 1 system (Roche). Oligonucleotide primer sequences for hamster FSHβ-and LHβ subunit were as shown in Table 1. For PCR analysis, sample cDNA was diluted 1:20 to 1:300, and PCR conditions, including Mg^{2+} and primer concentrations, anneal times, and extension times are summarized in Table 1. Standard curves for PCR analyses were generated using dilutions of a cDNA preparation of arbitrary unitage isolated from the pituitaries of adult hamsters in long-day cycle, and all data were normalized to the mass of total RNA in each sample. Unless otherwise noted, PCR of all samples was performed using triplicate reactions for 38 cycles, after which a melting curve analysis was performed to monitor PCR product purity (Table 1). In initial experiments, amplification of a single PCR product was confirmed by agarose gel electrophoresis and DNA sequencing.

Statistics

Treatment groups were compared with short-day controls using the Student-Newman-Kuels test or, in the case of unequal variance, Dunn method, with the program Sigmastat for Windows version 2.0 (Jandel Corporation, CA) for body and organ weights, serum FSH, and cell populations. Data for testosterone concentrations and FSHβ and LHβ subunit expression were log transformed and analyzed using GB Stat (Dynamic Systems Inc., Silver Spring, MD). The data are expressed as mean ± SEM, with n = 6–10 animals/group.

RESULTS

Body Weights

Body weights were reduced in short-day control to 78% of long-day control ($P < 0.001$). Hormone administration did not affect body weight compared with their corresponding short-day controls (Table 2).

Testicular Weights

Testicular weights (Table 2) from short-day animals were reduced ($P < 0.001$) to 5% of long-day control values. In response to FSH administration, testicular weights were increased 3-fold above short-day controls, to 17% of long-day controls ($P < 0.001$) (Table 2). Coadministration of FSH with flutamide had no further effects compared with FSH alone. Testicular weights were unaffected following flutamide-alone treatment and remained at short-day control values.

Prostate Weights

Prostate weights for short-day controls were reduced to 16% of long-day controls ($P < 0.001$; Table 2). FSH had no effect on prostate weights. Flutamide alone did not affect prostate weights compared with short-day controls; however, prostate weights were increased in FSH plus flutamide-treated hamsters.

Hormone Levels

Serum and testicular testosterone. Serum testosterone levels in short-day hamsters were not significantly reduced compared with long-day controls, but remained near the detection limit of the assay (Table 2). FSH alone or coadministered with flutamide did not alter serum testosterone levels compared with short- and long-day controls. Flutamide alone had no significant effect on serum testosterone levels (Table 2). Testicular testosterone levels in short-day controls were reduced to 5% of long-day control values ($P < 0.001$; Table 2). Exogenous FSH alone did not affect testicular testosterone levels. FSH in combination with flutamide or flutamide alone increased testicular testosterone to 10% of long-day values ($P < 0.01$).

Serum FSH. Serum FSH levels were reduced to the detection limit of the assay (3.2 ng/ml) in short-day controls (Table 2). Administration of flutamide did not alter serum FSH levels, which remained at short-day control values.

FSHβ and LHβ Subunit mRNA Expression Levels in Hamster Pituitary Glands

Because serum FSH levels were not significantly different in short-day hamsters compared with long-day controls (probably as a result of poor assay sensitivity), pituitary FSHβ subunit expression was measured as an indicator of gonadotropin suppression.

FSHβ subunit expression was reduced to ~30% ($P < 0.01$) in short-day control hamsters compared with long-day controls (Fig. 1). FSHβ subunit expression was undetectable following administration of FSH alone or in combination with flutamide ($P < 0.01$). FSHβ subunit expression levels were similar to short-day controls in hamsters receiving flutamide alone (Fig. 1).

LHβ subunit expression was reduced to ~38% ($P < 0.05$) in short-day control hamsters compared with long-day animals (Fig. 1). Expression levels following hormone treatment remained at short-day expression levels, except in flutamide-treated rats, where a 2-fold increase was observed, although this did not achieve significance.

Cell Populations

Sertoli cells. A reduction in Sertoli cell number to 67% was observed in short-day control hamsters ($P < 0.01$; Fig. 2) compared with long-day controls following 11 wk of photoinhibition. In response to FSH, regardless of flutamide administration, Sertoli cell number was increased to long-

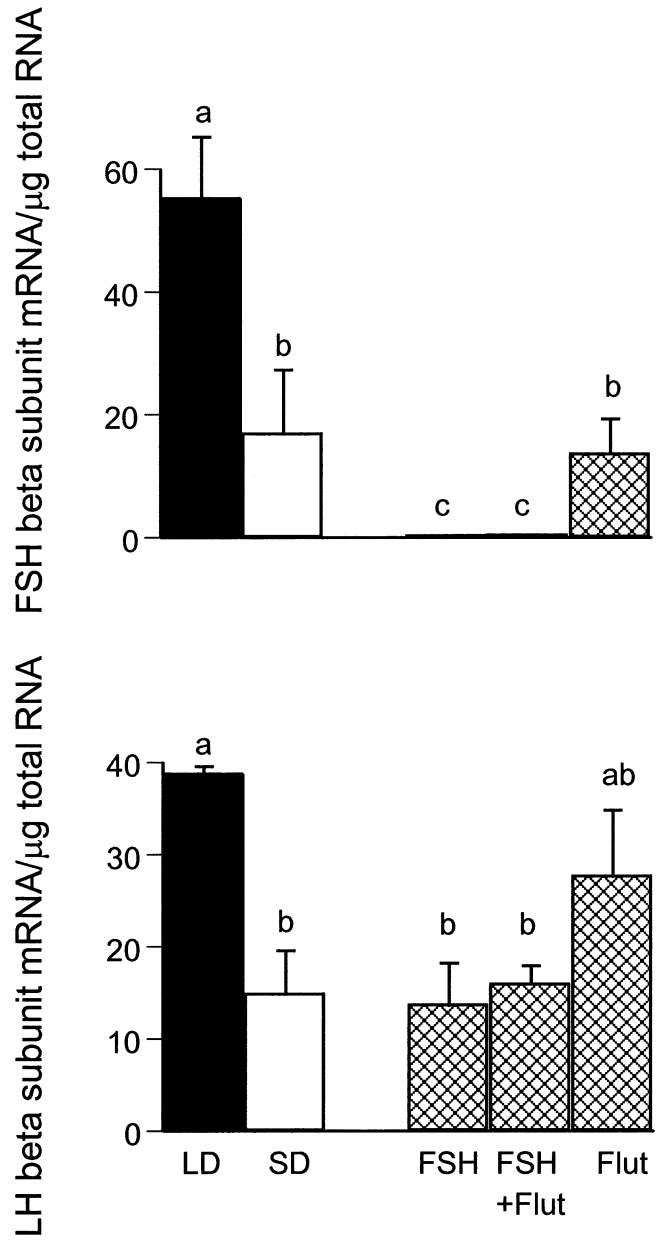

FIG. 1. Expression levels of FSHβ and LHβ subunit mRNA in the photostimulated (long day, LD) adult Djungarian hamsters that were exposed to short photoperiods (short day, SD) for 11 wk. Hamsters were given exogenous FSH (6 IU/day s.c.) alone or with flutamide (Flut, 10 mg/kg/day s.c.) for 7 days. Data are mean ± SEM; n = 6–7 hamsters/group. See text for significant differences.

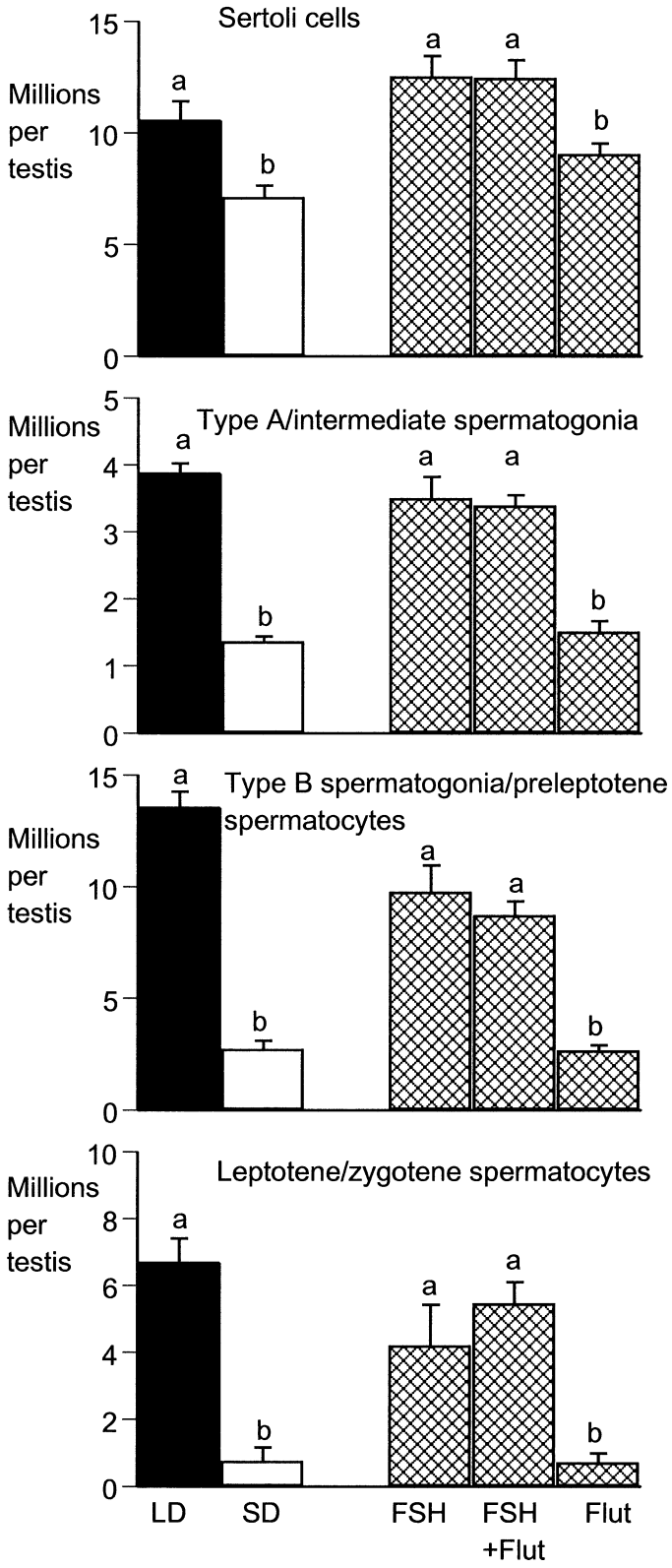

FIG. 2. The number of Sertoli cell, type A/intermediate spermatogonia, type B spermatogonia/preleptotene spermatocytes, and leptotene/zygotene spermatocytes (millions per testis) in the photostimulated (long day, LD) adult Djungarian hamsters that were exposed to short photoperiods (short day, SD) for 11 wk. Hamsters were given exogenous FSH (6 IU/day s.c.) alone or with flutamide (Flut, 10 mg/kg/day s.c.) for 7 days. Data are mean ± SEM; n = 10 hamsters/group. Letters denote significant differences between the treatment groups at or less than $P < 0.01$ (see text for specific information).

day control values. Flutamide treatment alone did not alter Sertoli cell number compared with short-day controls (Fig. 2).

Early germ cells. Compared with long-day controls, a reduction in type A spermatogonial, B spermatogonial/preleptotene spermatocyte, and leptotene/zygotene spermatocyte number to 35%, 19%, and 10%, respectively, in short-day control hamsters was observed (all $P < 0.001$; Fig. 2).

In response to FSH, with or without flutamide, type A spermatogonial, type B spermatogonial/preleptotene spermatocyte, and leptotene/zygotene spermatocyte number were increased to a mean of 90%, 70%, and 68% of long-day control, respectively (all $P < 0.01$; Fig. 2).

No difference was observed in any germ cell groupings

TABLE 3. The number of pachytene spermatocytes and round and elongated spermatids per testis (± SEM, n = 10/group) in photostimulated (long day) adult Djungarian hamsters that were exposed to short photoperiods (short day) for 11 weeks.

Treatment*	Pachytene spermatocytes (millions/testis)	Round spematids (millions/testis)	Elongated spermatids (millions/testis)
Long day control	57.9 ± 3.58[a]	159 ± 8.08[a]	152 ± 9.84
Short day control	0.48 ± 0.17[b]	0.003 ± 0.003[b]	0
+FSH	11.5 ± 1.34[c]	0.70 ± 0.57[b]	0
+Flut	0.72 ± 0.38[b]	0.09 ± 0.07[b]	0

* Treatment consisted of FSH (6IU/day, sc) alone or with flutamide (Flut, 10mg/kg/day, sc) for 7 days.
[a–c] Different superscripts indicate significant differences between the treatment groups at or less than $P < 0.01$.

in response to flutamide alone, with all groups remaining at short-day levels.

Later germ cells. Less than 1% of the pachytene spermatocytes remained in short-day controls compared with long-day control ($P < 0.01$; Table 3), while round spermatids were not detected in all short-day control hamsters except in the case of one animal. No elongated spermatids were observed.

In response to FSH treatment, with or without flutamide, pachytene spermatocyte numbers increased to 20% of long-day control ($P < 0.001$; Table 3), while flutamide alone had no significant effects on their population. In response to FSH treatment, with or without flutamide, round spermatid numbers were not affected. As expected, no elongated spermatids were observed after hormone replacement because 7 days is not long enough for the germ cells present in short-day animals to become elongated spermatids. Prolonged administration of FSH (e.g., 21 days) would be required to study maturational effects of FSH on these germ cell types.

DISCUSSION

In this study, we sought to establish the effect of FSH, in the presence of an antiandrogen, on the reinitiation of spermatogenesis in the adult photoinhibited Djungarian hamster and have shown that FSH exclusively regulates the reinitiation of spermatogenesis in this model. The optical disector stereological method has been used for the first time to quantify hamster Sertoli and germ cell populations. This study demonstrates that the 95% decrease in testis weight after 11 wk of photoinhibition, as a result of gonadotropin suppression, is attributable to significant decreases in both the Sertoli and germ cells populations, with spermatogenesis being arrested at the level of primary spermatocytes. Administration of FSH for 7 days increased testis weight 3-fold above short-day controls and was associated with an increase in both Sertoli and early germ cell populations, most notably spermatogonia, although FSH did provide some support for spermatocyte and spermatid development. There have been no previous quantitative reports of germ cell populations for the Djungarian hamster, but qualitatively normal spermatogenesis has been reported in response to FSH, in spite of low intratesticular testosterone levels [12], suggesting spermatogenesis is highly dependent on FSH.

This study has shown that the adult Sertoli cell population can be hormonally regulated as evidenced by a 33% reduction in the Sertoli cell population after photoinhibition and a restoration to long-day values within 7 days of exogenous FSH treatment. Furthermore, androgen action was not important for the FSH regulation of Sertoli cells number, as there was no change in their numbers after androgen blockade. The general view of adult mammalian Sertoli cells is that their population is fixed and unmodified by hormones. This view largely derives from reports that Sertoli cells neither divide in adult or hypophysectomized or hypophysectomized-hormone-treated rats [27] nor degenerate after hypophysectomy [28]. However, fluctuations in Sertoli cell number in seasonal breeders [29] have been reported for the golden hamster [30, 31], red deer [32], stallion [33–35], and soay ram [36], although significant differences were only observed in stallion studies. It is possible that, with the use of modern stereological techniques to quantify cell numbers, reanalysis of these models would show significant changes in Sertoli cell numbers similar to that seen in this study using Djungarian hamsters.

To assess Sertoli and germ cell numbers, this study has used a modern stereological approach, which is an unbiased and assumption-free method applicable to the assessment of nonspherical particles, such as the nuclei of Sertoli cells, spermatogonia, and elongated spermatids [19]. Tissue distortion measurements for Bouins-fixed methacrylate-processed tissue have been made in our laboratory on a number of occasions [5, 20], with negligible distortion observed, and thus, cell estimates were not adjusted. The mechanisms by which Sertoli cell populations retract and expand in this model remain unknown; however, mediation of seasonal testicular regression by apoptosis has been reported in the Djungarian hamster [37, 38]. Cells undergoing apoptosis in this model have not been identified; however, apoptotic spermatogonia and spermatocytes have been reported in the photoinhibited Syrian hamster [39].

To identify sites of hormone action on the spermatogenic process, the following issues require consideration. First, the approach depends on the morphological classification of germ cells, which in turn is partly based on cell-association patterns (i.e., staging), of which there are 12 stages in the Djungarian hamster [22]. Staging is unreliable in the photoinhibited hamster due to the severe regression and the lack of specific markers to allow morphological discrimination of type A spermatogonial subtypes from intermediate and type B spermatogonia and preleptotene spermatocytes. Hence, germ cells were classified into broader categories based on their morphological characteristics without any reference to staging. The limitation of pooling germ cells into broader categories is that effects on particular germ cell subpopulations can be masked. Second, a steady state of germ cell development under the influence of each treatment cannot be established over the 7-day period of this study. Therefore, changes in a particular germ cell population may have occurred as a result of effects on germ cell types arising up to 7 days earlier in the spermatogenic process. It has been assumed in the current study that the rate of germ cell development is not altered by gonadotropin withdrawal in the photoinhibited adult hamster, as has previously been demonstrated in the gonadotropin-withdrawn adult rat [40].

This study has shown that photoinhibition suppresses spermatogonial and primary spermatocyte numbers to 25% and 10%, respectively, of long-day control levels, with cells beyond early pachytene spermatocytes being almost eliminated. These data are in broad agreement with the pattern of spermatogonial suppression observed following gonadotropin withdrawal in monkeys and men in which cell numbers were assessed using stereological methods [7–10, 41].

In contrast, in the long-term gonadotropin-withdrawn rat model, spermatogonial numbers were only reduced to 50% of control, with spermatogenesis being arrested at midspermiogenesis [5, 6]. These data suggest that spermatogenesis in the gonadotropin-withdrawn Djungarian hamster model is more similar to the gonadotropin-withdrawn monkey and human than to the rat.

It is clear that FSH plays a key role in the reinitiation of spermatogenesis in the photoinhibited hamster, primarily through the prompt normalization of spermatogonial number within 7 days, but also by providing some support for the maturation of spermatocytes as evidenced by a partial restoration of spermatocyte number compared with long-day control animals. This study also shows that a small amount of testosterone in the photoinhibited hamster testis provides no support for developing germ cells, as evidenced by a lack of differences in germ cell populations after flutamide administration. These data are consistent with hypogonadal mice expressing transgenic FSH, which showed that FSH is important for Sertoli and spermatogonial development, with testosterone having little effect on their development [42–44]. Similar results have been reported in rats following chronic gonadotropin withdrawal, showing that FSH, but not testosterone, is important for restoration of the initial phases of spermatogenesis [6]. In regard to the spermatogonial subpopulations affected by FSH in this model, it is likely that the FSH-induced rise in type B spermatogonial/preleptotene spermatocyte number may have been due to the rise in type A spermatogonial mitosis or survival or both. In contrast, FSH did not increase round spermatid numbers above that of short-day control because it takes more than 7 days for spermatogonia to progress to round spermatids. Given that the duration of one cycle of the seminiferous is 7.9 days in the adult Djungarian hamster spermatogenesis epithelium [22] and takes 35 days for a spermatogonium to become an elongated spermatid, FSH would need to be administered for 21 days to photoinhibited hamsters to examine FSH effects on spermatid maturation. Round spermatids in other rodent models have been shown to be testosterone dependent [42, 45, 46], with even a small amount of testosterone playing a role [18, 47].

Even though data from this study and that of others [12, 14] demonstrate that FSH is needed for Djungarian hamster spermatogenesis, it cannot be ruled out that higher levels of testicular testosterone may reinitiate the spermatogenic process in a similar manner. It is clear from rat studies that testicular testosterone levels of 10–20% of normal are sufficient to maintain spermatogenesis [48–50]; however, similar studies need to be performed in the Djungarian hamster model to establish this point.

Germ cell development is well known to be reliant on the number of Sertoli cells [51, 52]. Because Sertoli cell number alters in this model, it is interesting to determine whether the Sertoli cell has a reduced capacity to nurture the developing germ cells following photoinhibition. The data suggest that Sertoli cells following photoinhibition support less than 3% of the photostimulated germ cell capacity (spermatogonia to elongated spermatids). This is largely attributed to the ablation of the most abundant germ cells, i.e., spermatids, of which there are about ~300 million per testis. More specifically, for early germ cells (spermatogonia to primary spermatocytes), Sertoli cells only support a fifth of the number seen in their long-day control counterparts. The mechanism(s) by which Sertoli cells lose their ability to support germ cells as a consequence of hormonal deprivation remains uncertain; however, loss of Sertoli cell function, e.g., degradation of the blood testis barrier, may play a role [11].

Previous reports show that exposure of photostimulated hamsters to photoinhibition suppresses spermatogenesis as a consequence of LH/testosterone and FSH suppression [11–13]. Consistent with this proposition, testicular testosterone levels in short-day hamsters were decreased to only 5% of long-day levels, similar to other reports. Serum testosterone in this study was not significantly altered following exposure to short photo periods, as also observed by others [53, 54]. FSH levels were also decreased in photoinhibited hamsters; however, due to the poor sensitivity of the FSH assay (only 40% that of photostimulated controls), the extent of this decline could not be accurately assessed. Therefore, pituitary FSHβ mRNA levels were also monitored and revealed after photoinhibition that FSHβ mRNA were significantly reduced compared with photostimulated hamsters. This argues against the possibility that the short photoperiod-exposed hamsters were spontaneously recrudescing. In the presence of flutamide, testicular testosterone levels were increased, as were prostate weights, in the FSH plus flutamide-treated group compared with photoinhibited controls, which suggests that the release of endogenous LH may have been stimulated.

In conclusion, this study demonstrates, for the first time using modern stereological techniques, that FSH, in the presence of an antiandrogen, regulates the reinitiation of spermatogenesis in the photoinhibited Djungarian hamster. The adult Sertoli cell population in this model retracts (during photoinhibition) and expands promptly to normal (within 7 days) in response to hormonal cues, with Sertoli cell number being dependent on FSH for restoration of their population. This naturally occurring model now provides a unique opportunity to understand the mechanism (apoptotic and/or proliferative) by which FSH regulates Sertoli and germ cell development in the adult animal.

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