The cycle duration of the seminiferous epithelium remains unaltered during GnRH antagonist-induced testicular involution in rats and monkeys

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Abstract

Although the gonadotropic control of the spermatogenic process is well established, the endocrine regulation of the timing and kinetics of germ cell development has received little attention. We found previously that the administration of a GnRH antagonist (ANT) over a period of 25 days could retard spermatid development and slightly prolong cycle length in intact adult cynomolgus monkeys (*Macaca fascicularis*). The aim of the present study was to investigate the effects of extended exposure to ANT on the duration of the cycle of the seminiferous epithelium in the monkey. Additionally, the duration of spermatogenesis was studied in the ANT-exposed rat model. In experiment 1, monkeys were given either saline or ANT ($n=6/\text{group}$) and on day 30 all animals received a single injection of 5-bromodeoxyuridine (BrdU) to label S-phase germ cells. Testicular biopsies were taken on days 39, 43, 47 and 51 (end of treatment) for BrdU localization and flow cytometric analysis. ANT treatment suppressed hormone levels, reduced testis size by $>70\%$ and severely

impaired germ cell production. Despite these alterations, cycle duration remained unchanged at all time-points compared with controls (10.12 ± 0.15 days vs 10.16 ± 0.44 days). In experiment 2, adult male Sprague–Dawley rats ($n=15/\text{group}$) received either vehicle (VEH) or ANT for 14 days and received BrdU injection on day 2. Cycle duration was found to be shorter in the ANT-treated group (12.45 ± 0.09 days) than in the control group (12.75 ± 0.08 , $P<0.05$). As spermatogenic cycle length in this control group was longer than that of our historical controls (range: 12.37–12.53 days), experiment 2 was repeated ($n=10/\text{group}$). In experiment 3, cycle duration was 12.51 ± 0.02 for VEH and 12.46 ± 0.05 for the ANT-treated group ($P>0.05$) in both species. We concluded that the duration of the cycle of the seminiferous epithelium in monkeys and rats is independent of gonadotropins but is rather regulated by the spermatogenic tissue itself.

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Introduction

Spermatogenesis is a complex process in which germ cells, supported by Sertoli cells, undergo mitotic and meiotic divisions and differentiation to produce elongated spermatids. Follicle-stimulating hormone (FSH) and testosterone released from the Leydig cells under the influence of luteinizing hormone (LH) are the key regulators of the spermatogenic function of the testis (for review see Weinbauer & Nieschlag 1996, 1998). Although there is controversy regarding the relative importance of FSH and LH/testosterone for spermatogenesis (Kumar *et al.* 1997, Tapanainen *et al.* 1997, Moudgal & Sairam 1998, Sairam *et al.* 1998, Weinbauer & Nieschlag 1998), it is clear that both hormones are needed for quantitatively normal

spermatogenesis. Selective suppression of LH and FSH secretion by means of administration of potent gonadotropin-releasing hormone (GnRH) antagonists leads to testicular involution depending on the length of the hypogonadotropic condition (Weinbauer & Nieschlag 1992).

In the primate, withdrawal of gonadotropin support to the testis specifically induces a marked drop in the number of B-spermatogonia and, with time, the progressive loss of the more advanced germ cells owing to lack of supply of precursor cells (Weinbauer *et al.* 1998, Zhengwei *et al.* 1998a,b). In the rat, the earliest sign of gonadotropin deficiency is the appearance of morphologically abnormal germ cells in stage VII or VIII of spermatogenesis (Russell & Clermont 1977, Sinha-Hikim *et al.* 1997), but germ cells in other stages are also affected at later time-points

(El Shennawy *et al.* 1998). Although many studies have addressed the morphological and functional consequences of hypogonadotropism on the seminiferous epithelium in several species, the issue of whether the duration of the spermatogenic process is under endocrine control has received only little attention. Clermont & Harvey (1965) reported that hypophysectomy slightly but not significantly prolonged the duration of the spermatogenic process from 12.9 to 13.2 days and hormone replacement reversed this minor effect. In two men, one treated with progesterin and the other one with human chorionic gonadotropin, spermatogenic cycle length was similar to that of untreated men (Heller & Clermont 1964). More recently, we observed a specific delay in spermatid development resulting in a slight but non-significant prolongation of cycle duration in monkeys made hypogonadotropic for 25 days from 9.8 days to 10.8 days (Weinbauer *et al.* 1998). The latter study was based on studying 5-bromodeoxyuridine (BrdU)-labeled germ cells whereas the former two investigations were based upon ^3H -thymidine incorporation.

The feasibility of using BrdU injection to label and trace germ cells has been demonstrated in laboratory and feral rats (Rosiepen *et al.* 1994, 1995), in the blue fox (Clausen *et al.* 1992) and in macaques (Rosiepen *et al.* 1997, Weinbauer *et al.* 1998), and the values obtained for the duration of one seminiferous cycle were nearly identical to those obtained by radioactive and other approaches (Rosiepen *et al.* 1994, 1997). The purpose of the present studies was twofold: (1) to investigate whether extended exposure (51 days) of GnRH antagonist (ANT)-induced gonadotropin deficiency affects spermatogenic cycle length in the monkey and (2) to assess the effects of gonadotropin deficiency on spermatogenic cycle length in the rat model using the BrdU approach.

Materials and Methods

Animals

Twelve adult intact male cynomolgus monkeys (*Macaca fascicularis*), weighing 4.44–9.00 kg, were caged individually. Fifty adult male Sprague–Dawley rats, weighing 390–460 g ($n=30$, experiment 2) and 250–300 g ($n=20$, experiment 3) were obtained from Charles River (Sulzfeld, Germany) and were kept in groups of three per cage. All animals were maintained under constant environmental conditions and were fed with standard pellet food with free access to tap water. Monkeys were also supplemented with fresh fruits. The study was undertaken in accordance with the German Federal Law on the Care and Use of Laboratory Animals.

Experiment 1 (Fig. 1) Monkeys were randomly allocated to daily s.c. injections of either VEH (5.25% glucose in saline; $n=6$) or the ANT cetorelix (450 µg/kg, $n=6$;

Figure 1 Experimental design for experiment 1 in monkeys (1), experiment 2 in rats (2) and experiment 3 in rats (3). Bx=testicular biopsy.

T Reissmann, ASTA Medica, Frankfurt, Germany) throughout a period of 51 days. The dose of cetorelix was based on previous experiments in which the animals showed antigonadotropic and antitesticular effects (Weinbauer *et al.* 1998). On day 30, all animals received a single i.v. injection of BrdU (Sigma Chemical Co., St Louis, MO, USA) at a dose of 590 mg/m² BrdU corresponding to 33 mg/kg (Rosiepen *et al.* 1997, Weinbauer *et al.* 1998). Testis biopsies were performed on days 39, 43, 47 and 51 on alternate sides. These time-points were selected in order to cover spermatocyte: spermatid transitions and round spermatid development (Weinbauer *et al.* 1998). Part of the biopsied tissue was fixed in Carnoy's solution for localization of BrdU and part was frozen for flow-cytometric analysis. All experiments were performed with animals under ketamine hydrochloride sedation (8 mg/kg; Ketamine, Parke Davis, Munich, Germany) and biopsies were performed under pentobarbital anesthesia. Blood was collected from the saphenous vein for hormone analysis on days -18, 0, 5, 12, 19, 26, 30, 39, 43, 47 and 51. Body weights were also recorded on these occasions.

Experiment 2 (Fig. 1) Rats ($n=15$ /group) were randomly allocated to daily s.c. injections of either VEH (5.25% glucose in saline) or cetorelix (112.5 µg/kg) throughout a period of 14 days. The dose and duration of ANT were determined in a dose-finding study aimed at identifying a dose that suppresses gonadotropin secretion but preserves the stage-specific organization of the germinal epithelium over the length of one spermatogenic

cycle (data not shown). On study day 2, all animals received a single i.p. injection of BrdU (100 mg/kg). The left testis was removed 3 h after BrdU administration and the right testis at study termination. Tissue was fixed for 24 h in Bouin's solution for localization of BrdU. Body and testes weights were recorded on days 2 and 14 of the experiment. Blood samples were collected from the retro-orbital sinus on days 1, 3, 6 and from the trunk on day 14 for analysis of serum testosterone and FSH.

Experiment 3 (Fig. 1) Since the duration of the seminiferous cycle of the VEH group in experiment 2 was higher than in historical controls (Rosiepen *et al.* 1994, 1995), this experiment was repeated ($n=10/\text{group}$) using rats with lower body weights (250–300 g). Body and testes weights were recorded on days 2 and 14. Blood samples were taken on experimental days 1, 7 and 14 for the determination of serum testosterone and FSH.

Immunohistochemical localization of BrdU-labeled germ cells

Fixed testicular biopsy material was dehydrated and embedded in paraffin by routine procedures. Carnoy-fixed sections from monkeys were incubated with 4 M HCl for 20 min at room temperature to hydrolyse the DNA allowing access of BrdU antibody and staining of the acrosome. Bouin-fixed rat sections were incubated with 1 M HCl for 7 min at 60 °C in a microwave oven (H2500; BioRad, Muenchen, Germany) followed by digestion with 0.1% trypsin (Sigma, Deisenhofen, Germany) for 10 min at room temperature. Immunocytochemical localization of BrdU by immunogold-silver staining and staining with periodic acid and Schiff's reagent for identification of spermatogenic stages was performed as previously reported in detail (Rosiepen *et al.* 1994, 1997, Weinbauer *et al.* 1998). Silver enhancement lasted for 11 min and sections were counterstained with hematoxylin, dehydrated and mounted in Vitro Clud.

Determination of the duration of the spermatogenic cycle

To determine the duration of the cycle of the seminiferous epithelium in the rat model, the procedure previously established was applied (Rosiepen *et al.* 1994). The duration of cycle of the seminiferous epithelium in the monkey was assessed as previously described (Rosiepen *et al.* 1997, Weinbauer *et al.* 1998) with some modifications.

Stage frequencies are based on previous studies in the cynomolgus monkey (Fouquet & Dadoune 1986, Weinbauer *et al.* 1998) and ANT does not alter stage frequencies (Weinbauer *et al.* 1998). The average duration of the spermatogenic cycle in the cynomolgus monkey is 9.8 days (Weinbauer *et al.* 1998) and the relative duration of each stage was calculated using 9.8 days as reference value. Since the amount of biopsy tissue was not sufficient to precisely determine the frequency of the spermatogenic

stages and the percentage of stages containing BrdU-positive germ cells, the following procedure was used. The duration of the cycle was estimated on the basis of stage frequency and BrdU-staining frequency between any two time-points. If the BrdU label was seen in a particular stage, it was arbitrarily assumed that the labeled cells occupied 50% of that stage. The duration was defined using the formula: $9.8 \pm (9.8/100(D))$: $\pm$ means that the term in brackets is added if the cells are lagging behind or subtracted if cells had traveled faster; D =distance across stages traveled by the BrdU-labeled cell between two time-points. For example, if at one time-point the label had progressed into stage VIII (relative duration: 0.77 days) and in the next cell layer into stage VI (relative duration: 1.11 days) at the second time-point, calculations were as follows: $9.8 \pm ((9.8/100) (0.38 + 1.31 + 0.55)) = 10.01$ days (duration of one cycle of the seminiferous epithelium). $D=2.24$ in this particular example. 0.38 and 0.55 represent 50% of stages VIII and VI, and 1.31 is the relative duration of stage VII. Cycle duration was calculated for any possible combination between the four time-points (see Fig. 5).

Quantification of testicular cell populations by DNA flow cytometry in monkey testis

In order to assess the spermatogenic status, monkey germ cells were released from the testicular biopsies and analysed as reported (Krishnamurthy *et al.* 1998). Testicular cells were stained with propidium iodide and analyzed using a Coulter flow cytometer EPICS XL (Coulter, Krefeld, Germany) equipped with 15 mW argon-ion laser at an excitation wavelength of 488 nm. The fluorescence signals of the propidium iodide-stained germ cells were collected at 620 band pass filter (605–635 nm). Cell populations analysed were HC cells (elongated spermatids), 1C cells (round spermatids), 2C cells (spermatogonia, Leydig cells, Sertoli cells, other somatic cells, secondary spermatocytes) and 4C cells (cells during mitotic or primary meiotic division after DNA synthesis and before division, spermatogonia and somatic cells during the G2 phase and primary spermatocytes).

Testicular volumes and serum hormone concentrations

The length and width of the monkey testes were measured with the help of Vernier callipers and testicular volume was calculated as described (Weinbauer *et al.* 1998). Serum samples were assayed for testosterone and FSH concentrations using RIA as described (Brinkworth *et al.* 1995, Chandolia *et al.* 1991a). Limit of detection, and inter- and intra-assay coefficients of variation for testosterone assays were 0.67 nmol/l, 7.5% and 9.2% respectively. Assay detection limit was 0.78 mg/l and intra- and interassay variations were 7.3% and 8.4% respectively for the FSH RIA.

Figure 2 Testis size and serum concentrations of testosterone in monkeys receiving vehicle (open circles, $n=6$) or the GnRH antagonist cetrorelix (closed circles, $n=5$) throughout 51 days. Asterisks denote significant differences ($P<0.05$) between groups. Values represent means $\pm$ S.E.M.

Statistical evaluation

Data are presented as means $\pm$ S.E.M. Two-way ANOVA was applied for the analysis of data in experiment 1 and for hormone values in experiments 2 and 3. Student's *t*-test was applied for analysis of differences in stage/staining frequencies, cycle duration of the seminiferous epithelium, body and testis weights in experiments 2 and 3. Probability values less than 0.05 were considered significant.

Results

Body weight, testis volume/weight and spermatogenic status

Mean testicular volumes of VEH-treated monkeys ranged between 32.4 ± 2.5 ml and 26.3 ± 2.6 ml whereas, in ANT-treated animals, testes significantly declined to 12.9 ± 1.3 ml by day 39 and remained low (12.9 – 13.8 ml) thereafter (Fig. 2). One animal from the ANT-exposed group did not respond and was excluded from the analysis.

Body weights were reduced by 10–12% of baseline in the ANT-treated group (data not shown).

Flow cytometric analysis revealed a 50% reduction ($P<0.05$) of haploid germ cell numbers (data not shown). The 4C:2C ratio was lowered two- to threefold ($P<0.05$) indicating that ANT suppressed spermatogonial proliferation and primary spermatocyte formation. Meiotic transformation remained unaffected ($P>0.05$) as seen from the 1C:4C ratios.

Body weights in rats were lowered on day 14 in the ANT-treated groups in experiments 2 and 3 (Figs 3 and 4). Testicular weights showed a significant decline on day 14 in the ANT-treated group with reference to VEH treatment in experiments 2 and 3 (Figs 3 and 4).

Serum hormone concentrations

Mean testosterone concentrations in VEH-treated monkeys varied from 20–40 nmol/l and dropped from a mean pretreatment value of 36.2 ± 8.5 to 6.0 ± 0.8 on day 5 and to nadir values of 2.9 ± 0.2 nmol/l in the ANT-treated group (Fig. 2). In experiments 2 and 3 (rat), serum concentrations of testosterone and FSH were significantly reduced during ANT exposure (Figs 3 and 4).

Localization of BrdU and duration of the spermatogenic cycle

Sertoli cells containing BrdU were not encountered. Occasionally a peritubular cell or an interstitial cell was BrdU positive. In the monkey testis, depending on the time after BrdU application, spermatocytes and spermatids contained BrdU label in various spermatogenic stages in both groups. Among rats, BrdU-positive germ cells at the second time-point were spermatocytes that progressed into stages VII, VIII, IX and X of the spermatogenic cycle (data not shown). Hence, only these stages were considered for the determination of the length of the spermatogenic cycle in the rat.

Monkeys Calculation of the duration of the spermatogenic cycle in the cynomolgus monkey yielded comparable values irrespective of the time-points used (Fig. 5). The intra-animal variation in cycle duration was $3.0 \pm 0.7\%$, interanimal variation was $3.5 \pm 0.7\%$. For calculation of the cycle duration for each animal, the mean value of the six combinations between time-points was used. The duration of one cycle of the seminiferous epithelium was 10.16 ± 0.44 days in the VEH group and 10.12 ± 0.15 days ($P>0.05$) in the ANT group.

Rats Neither stage frequencies nor the percentage of tubules at a particular stage containing BrdU-positive cells (BrdU-staining frequency) were consistently different between VEH and ANT groups on day 2 in experiments 2 and 3 (Table 1, Fig. 6). On day 14, the frequency of stage VIII seminiferous tubules decreased significantly whereas

Figure 3 Body and testis weight, and serum hormone concentrations in rats from experiment 2. Animals ($n=15/\text{group}$) received vehicle (open bars and circles) or the GnRH antagonist cetrorelix (solid bars and circles) throughout 14 days. Asterisks denote significant differences ($P<0.05$) between groups. Values represent means $\pm$ S.E.M.

Figure 4 Body and testis weight, and serum hormone concentrations in rats from experiment 3. Animals ($n=10/\text{group}$) received vehicle (open bars and circles) or the GnRH antagonist cetrorelix (solid bars and circles) throughout 14 days. Asterisks denote significant differences ($P<0.05$) between groups. Values represent means $\pm$ S.E.M.

the frequency of stage IX tubules was significantly higher (Table 1). With regard to BrdU-staining frequencies in experiment 2, significantly more tubules in stage IX and X

contained BrdU-labeled cells in the ANT group on day 2 and this effect became more pronounced by day 14 (Fig. 6). The BrdU label had progressed into stage X in

Figure 5 Duration of the cycle of the seminiferous epithelium in monkeys receiving vehicle (open bars, $n=6$) or the GnRH antagonist cetrorelix (solid bars, $n=5$). Bx=testicular biopsy taken on day 39 (Bx1), day 43 (Bx2), day 47 (Bx3) and day 51 (Bx4) of GnRH antagonist administration. Calculation of the duration is based on two time-points and all possible combinations were used (for example, Bx1–4 means that data from biopsy 1 and 4 were used). Differences between the treatment groups were not significant ($P>0.05$). Values represent means $\pm$ S.E.M.

one ANT-treated animal and into stage IX in only four VEH-treated animals. This difference resulted in a significant decrease of the duration of the spermatogenic cycle in the ANT group, i.e. 12.75 ± 0.08 days (VEH) vs 12.45 ± 0.09 days ($P<0.05$).

No significant difference could be detected for BrdU-staining frequencies on day 2 in experiment 3 (Fig. 6). By

day 14, only a slight but non-significant reduction of the frequency of stage VII tubules containing BrdU label was observed. The corresponding duration of the spermatogenic cycle was nearly identical for both groups (12.51 ± 0.02 days for VEH group and 12.46 ± 0.05 days ($P>0.05$) for ANT group).

Discussion

The present study was undertaken to investigate the effects of gonadotropin deficiency, caused by the administration of cetrorelix, a highly potent antagonist of GnRH (Weinbauer & Nieschlag 1993), on the duration of the cycle of the seminiferous epithelium in the adult monkey and the rat. Monkeys were exposed to ANT throughout a period of 51 days. This treatment length covers approximately five spermatogenic cycles (Weinbauer *et al.* 1998). Although ANT treatment markedly reduced testis size and affected the spermatogenic process as revealed by quantitative flow-cytometric analysis of testicular cells, the overall duration of the spermatogenic cycle was not altered either in comparison to VEH-treated animals or as a function of treatment length. In a previous investigation on the same animal species, ANT exposure for 25 days prolonged the length of the cycle of the seminiferous epithelium by about 1 day (9.8 days vs 10.8 days, Weinbauer *et al.* 1998). It was assumed that if this difference was truly caused by gonadotropin deficiency, prolonging the period of the hypogonadotropic condition would further reduce spermatogenic cycle length. Hence we suspected from our previous data that prolonged gonadotropin deficiency might alter the timing of germ cell development and the observation period was approximately doubled and several time-points for determining the spermatogenic duration were used in the present investigation. In the light of the present findings however, i.e. the absence of any indication of an alteration of spermatogenic cycle length, it would appear that the duration of the cycle of the seminiferous epithelium in the primate is not under the control of the pituitary hormones LH and FSH.

The first investigation in the rat model (experiment 2) revealed a significant acceleration (12.75 days vs 12.45 days) of the spermatogenic cycle induced within 14 days of ANT administration. Exposure of rats to 14 days of ANT treatment induces a quantitative reduction of germ cell production but spermatogenic stages can still be recognized (Chandolia *et al.* 1991b, Brinkworth *et al.* 1995, Gromoll *et al.* 1997). When extrapolated for the average time needed by a spermatogonium to develop into a mature testicular sperm, the difference is 1.2 days. These observations are at variance with those reported by Clermont & Harvey (1965). In that study hypophysectomy, if at all, increased spermatogenic cycle length. In the present study, the duration of the spermatogenic cycle in

Table 1 Stage frequencies (%) after GnRH antagonist treatment in the Sprague–Dawley rat

	VII	VIII	IX	X
Experiment 2				
Day 2				
VEH	16.9 $\pm$ 0.3	7.4 $\pm$ 0.2	3.0 $\pm$ 0.1	3.1 $\pm$ 0.1
ANT	17.0 $\pm$ 0.2	7.1 $\pm$ 0.2	3.3 $\pm$ 0.1	3.2 $\pm$ 0.1
Day 14				
VEH	16.6 $\pm$ 0.3	8.4 $\pm$ 0.2	3.0 $\pm$ 0.1	2.7 $\pm$ 0.1
ANT	16.4 $\pm$ 0.4	7.0 $\pm$ 0.2 ^b	3.5 $\pm$ 0.2 ^a	2.6 $\pm$ 0.1
Experiment 3				
Day 2				
VEH	16.6 $\pm$ 0.3	7.7 $\pm$ 0.2	3.1 $\pm$ 0.1	3.0 $\pm$ 0.2
ANT	16.8 $\pm$ 0.3	8.0 $\pm$ 0.2	3.2 $\pm$ 0.2	3.0 $\pm$ 0.1
Day 14				
VEH	17.6 $\pm$ 0.1	8.1 $\pm$ 0.2	3.4 $\pm$ 0.1	3.1 $\pm$ 0.1
ANT	17.8 $\pm$ 0.3	7.3 $\pm$ 0.2 ^b	3.8 $\pm$ 0.1 ^a	2.9 $\pm$ 0.1

^a $P<0.05$ and ^b $P<0.01$ vs VEH; values are mean $\pm$ S.E.M.; VEH: vehicle, ANT: GnRH antagonist. $n=15$ /group for experiment 2 and 10/group for experiment 3.

Figure 6 Percentage of tubules containing BrdU-labeled germ cells in various stages of the spermatogenic cycle in rats from experiment 2 (upper panels) and experiment 3 (lower panels) on days 2 and 14 of treatment with vehicle (open bars) or the GnRH antagonist cetrorelix (solid bars). Group size was 15 animals for experiment 2 and 10 animals for experiment 3. Asterisks denote significant differences ($P < 0.05$) between groups. Values represent means $\pm$ S.E.M.

the control groups was 12.75 days and this value deviated from those obtained for Sprague–Dawley control rats in previous studies in our laboratory (mean reference values ranged between 12.37 days and 12.53 days, Rosiepen *et al.* 1994, 1995). Moreover, alterations of the spermatogenic process are rather associated with an increase in cycle length (Rosiepen *et al.* 1995). Hemicastration is not likely to have caused this difference since it has been shown that the removal of one testis does not alter cycle duration (Rosiepen *et al.* 1994). Unlike in our previous studies, the rats used in the present investigation were substantially heavier. For that reason, the experiment was repeated and rats with body weights similar to those in previous investigations (Rosiepen *et al.* 1994, 1995) were used. In that study, the length of the spermatogenic cycle was virtually identical for VEH and ANT groups (12.51 days vs 12.46 days). We conclude from these data that, like in the primate, gonadotropin deficiency does not alter the duration of the cycle of the seminiferous epithelium in the rat model. An effect of age on cycle length cannot be ruled out from the present findings and it has been reported that

the first spermatogenic cycle during puberty is shorter than that during adulthood in mice and hamsters (Kluin *et al.* 1982).

In conclusion, the present study – based upon the BrdU-approach determination of the length of the spermatogenic cycle – demonstrates that the duration of the cycle of the seminiferous epithelium in the primate and rodent is not under the endocrine control of gonadotropic hormones. Although not addressed specifically in our studies, age-related effects on spermatogenic cycle duration are conceivable. Apparently, cycle duration is controlled within the seminiferous epithelium but the factors involved remain to be revealed.

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References

- Brinkworth MH, Weinbauer GF, Schlatt S & Nieschlag E 1995 Identification of male germ cell undergoing apoptosis in adult rat. *Journal of Reproduction and Fertility* **105** 25–33.
- Chandolia RK, Weinbauer GF, Simoni M, Behre HM & Nieschlag E 1991a Comparative effects of chronic administration of the non-steroid antiandrogens flutamide and casodex on the reproductive system of the adult male rat. *Acta Endocrinologica* **125** 547–555.
- Chandolia RK, Weinbauer GF, Fingscheidt U, Bartlett JMS & Nieschlag E 1991b Effects of flutamide on testicular involution induced by an antagonist of gonadotrophin-releasing hormone and on stimulation of spermatogenesis by follicle-stimulating hormone. *Journal of Reproduction and Fertility* **93** 313–324.
- Clausen OPF, Anderson Berg K, Kirkhus B, De Angelis P & Huitfeldt H 1992 BrdU incorporation studies for evaluation of spermatogenesis in the blue fox. *Cytometry* **13** 374–380.
- Clermont Y & Harvey SC 1965 Duration of the cycle of the seminiferous epithelium of normal, hypophysectomized and hypophysectomized-hormone treated albino rats. *Endocrinology* **76** 80–89.
- El Shennawy A, Gates RJ & Russel L 1998 Hormonal regulation of spermatogenesis in the hypophysectomized rat: cell viability after hormonal replacement in adults after intermediate periods of hypophysectomy. *Journal of Andrology* **19** 320–334.
- Fouquet JP & Dadoue JP 1986 Renewal of spermatogonia in the monkey (*Macaca fascicularis*). *Biology of Reproduction* **35** 199–207.
- Gromoll J, Wessels J, Rosiepen G, Brinkworth M & Weinbauer GF 1997 Expression of mitotic cyclin B1 is not confined to proliferating cells in the rat testis. *Biology of Reproduction* **57** 1312–1319.
- Heller CG & Clermont Y 1964 Kinetics of the germinal epithelium in man. *Recent Progress in Hormone Research* **20** 545–575.
- Kluin PHM, Kramer MF & de Rooij DG 1982 Spermatogenesis in the immature mouse proceeds faster than in the adult. *International Journal of Andrology* **5** 282–294.
- Krishnamurthy H, Jagetia GC & Jyothi P 1998 Radioprotective effect of zinc aspartate on mouse spermatogenesis: a flow cytometric evaluation. *Mutation Research* **401** 111–120.
- Kumar TR, Wang Y, Lu N & Matzuk MM 1997 Follicle stimulating hormone is required for ovarian follicle maturation but not male fertility. *Nature Genetics* **15** 201–204.
- Moudgal NR & Sairam M 1998 Is there a true requirement for follicle stimulating hormone in promoting spermatogenesis and fertility in primates? *Human Reproduction* **13** 916–919.
- Rosiepen G, Weinbauer GF, Schlatt S, Behre HM & Nieschlag E 1994 Duration of the cycle of the seminiferous epithelium, estimated by the 5-bromodeoxyuridine technique, in laboratory and feral rats. *Journal of Reproduction and Fertility* **100** 299–306.
- Rosiepen G, Chapin RE & Weinbauer GF 1995 The duration of the cycle of seminiferous epithelium is altered by administration of 2,5-hexanedione in the adult Sprague–Dawley rat. *Journal of Andrology* **16** 127–135.
- Rosiepen G, Arslan M, Clemen G, Nieschlag E & Weinbauer GF 1997 Estimation of the duration of the cycle of the seminiferous epithelium in the non-human primate *Macaca mulatta* using the 5-bromodeoxyuridine technique. *Cell and Tissue Research* **288** 365–369.
- Russell LD & Clermont Y 1977 Degeneration of germ cells in normal, hypophysectomized and hormone treated hypophysectomized rats. *Anatomical Record* **187** 347–366.
- Sairam MR, Dierich A, Monaco L, Fimia GM, Gansmuller A, Lemeur M & Sassone-Corsi P 1998 Targeted disruption of the FSH receptor leads to aberrant gametogenesis, hormonal imbalances causing infertility/reduced fertility. *Proceedings of the Endocrine Society's 80th Annual Meeting*, 24–27 June 1998; New Orleans, LA, USA. Abstract OR46–3.
- Sinha-Hikim AP, Rajavashisth TB, Sinha Hikim I, Lue Y, Bonavera JJ, Leung A, Wang C & Swerdloff RS 1997 Significance of apoptosis in the temporal and stage-specific loss of germ cells in the adult rat after gonadotropin deprivation. *Biology of Reproduction* **57** 1193–1201.
- Tapanainen JS, Aittomäki K, Min J, Vaskivuo T & Huhtaniemi IT 1997 Men homozygous for an inactivating mutation of the follicle-stimulating hormone (FSH) receptor gene present variable suppression of spermatogenesis and fertility. *Nature Genetics* **15** 205–206.
- Weinbauer GF & Nieschlag E 1992 LH–RH antagonists: state of the art and future perspectives. In *Peptides in Oncology I: LH–RH Agonists and Antagonists*, pp 113–136. Ed HG Höffken. Berlin: Springer-Verlag.
- Weinbauer GF & Nieschlag E 1993 Comparison of the antigonadotropic activity of three GnRH antagonists (Nal–Glu, antide and cetrorelix) in a non-human primate model (*Macaca fascicularis*). *Andrologia* **25** 141–147.
- Weinbauer GF & Nieschlag E 1996 Hormonal regulation of reproductive organs. In *Comprehensive Human Physiology*, pp 2231–2252. Eds R Gregor & U Windhorst. Berlin, Heidelberg: Springer-Verlag.
- Weinbauer GF & Nieschlag E 1998 The role of testosterone in spermatogenesis. In *Testosterone: Action, Deficiency, Substitution*, edn 2, pp 143–168. Eds E Nieschlag & HM Behre. Berlin, Heidelberg: Springer-Verlag.
- Weinbauer GF, Schubert J, Yeung CH, Rosiepen G & Nieschlag E 1998 Gonadotrophin-releasing hormone antagonist arrests premeiotic germ cell proliferation but does not inhibit meiosis in the male monkey: a qualitative analysis using 5-bromodeoxyuridine and dual parameter flow cytometry. *Journal of Endocrinology* **156** 23–34.
- Zhengwei Y, Wreford NG, Schlatt S, Weinbauer GF, Nieschlag E & McLachlan RI 1998a Acute and specific impairment of spermatogonial development by GnRH antagonist-induced gonadotrophin withdrawal in the adult macaque (*Macaca fascicularis*). *Journal of Reproduction and Fertility* **112** 139–147.
- Zhengwei Y, Wreford NG, Royce P, de Kretser DM & McLachlan RI 1998b Stereological evaluation of human spermatogenesis after suppression by testosterone treatment: heterogeneous pattern of spermatogenic impairment. *Journal of Endocrinology and Metabolism* **83** 1284–1291.

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