Effect of local heating of rat testes after suppression of spermatogenesis by pretreatment with a GnRH agonist and an anti-androgen

B. P. Setchell¹*, L. Plöen² and E. M. Ritzen¹

¹*Pediatric Endocrinology Unit, Karolinska Hospital, Stockholm, Sweden; and* ²*Department of Anatomy and Histology, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden*

The effects of local heating of rat testes, in which spermatogenesis had been suppressed with injections of a GnRH agonist and an anti-androgen, were examined. Although the detrimental effects of heating were not as marked as those found in the testes of non-injected rats, the testes in which spermatogenesis was suppressed also showed a significant reduction in mass, the number of spermatozoa, tubular diameter and the percentage of normal tubular cross-sections at day 35 after heating. The results indicate that heating has an effect on cells in the testis other than those shown to be most susceptible to

heat, namely pachytene spermatocytes and early spermatids, which were absent or markedly reduced in number when spermatogenesis was suppressed. The long-term effects of heating on the above parameters, as reported in a previous study, were also confirmed. However, in testes in which spermatogenesis was suppressed at the time of heating, there appeared to be no or a reduced long-term impairment of spermatogenesis, as determined by testis mass, the percentage of qualitatively normal tubules and epididymal sperm counts.

Introduction

The effects of high temperatures on spermatogenesis have been known for many years (for a review, see Setchell, 1998) and the most susceptible cells are thought to be pachytene spermatocytes and young spermatids. However, a single exposure of rat testes to 43°C for 30 min also causes a delayed secondary decrease in testis mass and number of spermatozoa, probably due to an effect on spermatogonial renewal or the number of stem cells, or a delayed effect on the Sertoli cells (Setchell *et al.*, 2001). This delayed response was reduced by treatment of the animals at the time of heating with a GnRH agonist and an anti-androgen, a treatment that is known to reduce damage to the testis caused by radiation or cytotoxic drugs (Meistrich and Kangasniemi, 1997; Shetty *et al.*, 2000; Hild *et al.*, 2001). The aim of the present study was to examine more fully the delayed response of the testis to short-term heating. The most susceptible cells were removed by suppressing spermatogenesis through pretreatment of rats with GnRH agonist and an anti-androgen before heating the testis, to determine whether other cells in the testis were susceptible to the effects of heat in the absence of the most susceptible types of germ cell. A preliminary report of these findings was presented to the Society for the Study of Fertility (Setchell *et al.*, 1999).

*Present address: Department of Anatomical Sciences, University of Adelaide, Adelaide 5005, Australia
Email: brian.setchell@adelaide.edu.au

Materials and Methods

Animals

Forty-eight male Sprague–Dawley rats were used, supplied by B and K Universal, Sollentuna, aged about 65 days and weighing about 350 g at the start of the experiment. The rats were allocated randomly into four groups: HZF, ZF, H (13 rats each) and C (nine rats). On day –21, rats in groups HZF and ZF were implanted with a 3.6 mg pellet of Zoladex (Zeneca, Macclesfield) and given the first of 12 daily s.c. injections of Flutamide (Schering-Plough, Stockholm; 20 mg kg⁻¹ body weight) in propanediol. On day 0, four rats in group ZF were anaesthetized, their testes and seminal vesicles (with the contents and the coagulating glands) removed and weighed, and the testes fixed by immersion in glutaraldehyde. The other rats were anaesthetized with pentobarbitone sodium (Apoteket, Stockholm; 50 mg kg⁻¹ body weight) and the testes of rats in groups HZF and H (as positive controls) were immersed in a waterbath at 43°C for 30 min. Although the testes of the rats in group HZF were smaller than normal, they were still in the scrotum, which extended normally in the warm bath, so that the testes were exposed to the same heat load as those of rats in group H. The testes of three other rats from the same group were removed on day –21 for other reasons, and the masses were included (Fig. 1).

On days 35 and 70, four rats in groups HZF and H and three rats from groups ZF and C were anaesthetized and their testes removed and weighed. Interstitial extracellular

Fig. 1. The mass of (a) one testis and (b) one epididymis of (○) control rats (group C), (□) rats treated with a Zoladex implant and 12 daily injections of Flutamide from day -21 to day -10 (65–76 days of age; group ZF), (●) rats in which the testes were heated by immersion in a waterbath at 43°C for 30 min on day 0 (86 days of age; group H), and (■) rats exposed to both hormone treatment and heating (group HZF). Arrowhead indicates administration of Zoladex; arrow indicates heating of testes. Four rats in group ZF were sampled on day 0; three rats in groups ZF and C were sampled on days 35, 70 and 105; four rats in groups HZF and H were sampled on days 35 and 70; and five rats in groups HZF and H were sampled on day 105. Testis masses for H35, H70, H105 and ZF35 ($P < 0.001$), ZF70 ($P < 0.01$) and HZF105 ($P < 0.05$) were all significantly less than corresponding C values; H70 ($P < 0.001$) and H105 ($P < 0.05$) were significantly less than corresponding HZF values; HZF35 ($P < 0.001$) and HZF105 ($P < 0.01$) were significantly less than corresponding ZF values; ZF105 was greater than ZF70 ($P < 0.05$). Epididymal masses for H105 ($P < 0.01$), H35, H70, HZF35, HZF70, HZF105, ZF35, ZF70 and ZF105 ($P < 0.05$) were all significantly less than corresponding C values; H35 was significantly greater than HZF35 ($P < 0.05$).

fluid was collected from the left testes by irrigating with 2 ml isotonic mannitol after opening the capsule, and the volume was estimated by measuring the sodium content by flame photometry, assuming that the concentration in the original fluid was like serum. The cells were then dispersed by forcing the parenchyma through a 21-gauge needle and centrifuging the dispersed cell suspension at 10000 *g* for 15 min to yield a cell sediment and a fluid supernatant consisting largely of tubular fluid, which were weighed separately. The right testis from each animal was fixed with glutaraldehyde by perfusion through the testicular artery on the surface of the testis when possible, or by immersion when the testes were too small for successful perfusion. Both epididymides and seminal vesicles were removed and weighed and the testis cells and the epididymides frozen for subsequent sperm counts. On day 105, samples were collected from five rats in groups HZF and H and three rats in groups ZF and C.

Cubes of parenchyma (about 1 mm³) were cut from near the centre of each fixed testis, post-fixed in osmium tetroxide, dehydrated and embedded in Agar 100 resin. Semi-thin sections (about 1 µm) were stained with buffered toluidine blue. Tubular cross-sections from each animal were used for measurement of tubular diameter, which was performed using a microscope fitted with a digital video camera and an image-analysis system. Tubular cross-sections were classified as normal if all generations of germ cells were present and there were no degenerating cells, or abnormal if some types of cell were missing or if degenerating cells were present. The sections were then examined at higher magnification to determine the most advanced stage of spermatogenesis in the abnormal tubular cross-sections.

Analyses

Spermatozoa in the testis cell fractions and epididymides were counted and extracellular fluid volumes calculated as described by Setchell *et al.* (2001). Data were analysed by ANOVA, followed by the least mean square test (Superanova, Abacus Concepts, Berkeley, CA) for individual comparisons of normally distributed data, by Mann–Whitney U-test for data such as number of spermatozoa, which were not normally distributed, or by chi-squared tests for the numbers of qualitatively normal and abnormal tubules.

Results

Tissue masses

As observed in the previous experiment (Setchell *et al.*, 2001), treatment of rats with GnRH agonist and anti-androgen caused a decrease in the size of the testes. In the present study, the testes decreased to about one-quarter of normal size between day -21 and day 0. The testes subsequently increased in mass, but were still smaller than those in rats in the control group on days 35 and 70, but were not different on day 105. When the testes of the

Table 1. Initial body weight (g) and daily body weight gain (g day⁻¹) of control rats (C), rats treated with a Zoladex implant and 12 daily injections of Flutamide from day -21 to day -10 (ZF), rats in which the testes were heated by immersion in a waterbath at 43°C for 30 min (H) on day 0, and rats exposed to both treatments (HZF)

	C	H	HZF	ZF
Initial body weight (day -21)	356 ± 3.8 (9)	355 ± 3.2 (13)	358 ± 3.0 (13)	357 ± 3.0 (13)
Daily gain (g day ⁻¹)				
Day -21 to day 0	4.66 ± 0.36 (9)	4.40 ± 0.21 (13)	3.58 ± 0.22 (13)**	3.66 ± 0.27 (13)**
Day 0 to day 35	3.49 ± 0.21 (9)	3.23 ± 0.12 (13)	3.45 ± 0.12 (13)	3.19 ± 0.22 (9)
Day 35 to day 70	1.86 ± 0.16 (6)	1.81 ± 0.17 (9)	2.15 ± 0.16 (9)*	2.53 ± 0.31 (6)*
Day 70 to day 105	0.95 ± 0.42 (3)	1.06 ± 0.24 (5)	1.28 ± 0.19 (5)	1.33 ± 0.47 (3)

Values are mean ± SEM. Values in parentheses are number of observations.

* $P < 0.05$, ** $P < 0.01$: ZF and HZF combined significantly different from C and H combined.

hormone-treated rats were heated, they increased in mass as the effects of the hormone treatment wore off. However, at day 35 after heating, the testes were significantly smaller than unheated testes from hormone-treated rats. This difference had disappeared by day 70 after heating, although at day 105, in group HZF (hormone treatment and heating) the testes were no larger than at day 70 and were again smaller than those in rats in group ZF (hormone treatment and no heating). At day 105, the testes of the rats in groups HZF and H were smaller than those of control rats, whereas the testes of the rats treated only with hormones were not, and were larger at day 105 than at day 70 (Fig. 1). As reported in the previous paper (Setchell *et al.*, 2001), heating the testes of non-hormone-treated rats caused a decrease in testis mass to about half the normal mass at day 35, with partial recovery at day 70, but no further improvement at day 105.

The mass of the epididymis was also reduced by hormone treatment, and remained lower than in control rats for the duration of the study. A similar result was observed in the group exposed to heat and hormone treatment. The epididymides of the rats exposed to heat were also smaller than those of rats in the control group on days 35, 70 and 105 after heating, but were not as small on day 35 as the epididymides of rats exposed to heat and hormone treatment (Fig. 1).

The mass of the seminal vesicles was not affected by heating the testes but was markedly reduced by hormone treatment. In the two hormone-treated groups, the mass of the seminal vesicles had recovered to control values by day 70, although there was a significant difference at day 105 between the combined values for these two groups (combined because heating had no effect) and combined values for the two groups not treated with hormones (Fig. 2).

Body weight was similar in all groups at the start of the experiment, and heating the testes had no effect on subsequent weight gain. However, as reported previously (Setchell *et al.*, 2001), the daily weight gain was initially significantly reduced in the hormone-treated groups, compared with that in the pooled C and H groups, but then showed a compensatory increase (Table 1), so that body weight was similar in

Fig. 2. The mass of both seminal vesicles, with coagulating glands and contents (g), of (○) control rats (group C), (□) rats treated with a Zoladex implant and 12 daily injections of Flutamide from day -21 to day -10 (65–76 days of age; group ZF), (●) rats in which the testes were heated by immersion in a waterbath at 43°C for 30 min on day 0 (86 days of age; group H), and (■) rats exposed to both treatments (group HZF). Arrowhead indicates administration of Zoladex; arrow indicates heating of testes.

all groups by the end of the experiment (data not shown).

Numbers of spermatozoa in testis and epididymis

In group HZF, the number of spermatozoa in the testis was lower than that in group ZF but higher than in group H at day 35. Similarly, the number of spermatozoa in the epididymis in group HZF was lower than that in group ZF at day 35; however, in contrast to numbers in the testis, which were higher than those in group H at day 35, the number of spermatozoa in the epididymis in group HZF was lower than in group H on day 35, but higher on days 70 and 105. In groups H, ZF and HZF, the number of spermatozoa in the

Fig. 3. Numbers of spermatozoa ($\times 10^6$) in (a) one testis and (b) one epididymis of (○) control rats (group C), (□) rats treated with a Zoladex implant and 12 daily injections of Flutamide from day -21 to day -10 (65–76 days of age; group ZF), (●) rats in which the testes were heated by immersion in a waterbath at 43°C for 30 min on day 0 (86 days of age; group H), and (■) rats exposed to both treatments (group HZF). Numbers of spermatozoa in the testis for H35, H70, H105 and HZF35 were significantly less than corresponding C values ($P < 0.05$); HZF35 and HZF70 were significantly different from corresponding ZF values ($P < 0.05$); H35 and H70 were significantly less than corresponding HZF values ($P < 0.05$). Numbers of spermatozoa in the epididymis for H, HZF and ZF groups were significantly less than corresponding C values ($P < 0.05$); all H values were significantly different ($P < 0.05$) from corresponding HZF values; HZF35 was significantly less than ZF35 ($P < 0.05$).

epididymis remained lower than that in the control group throughout the experiment (Fig. 3).

As reported previously (Setchell *et al.*, 2001), heating the testes of rats not pretreated with hormones caused a marked

Fig. 4. Volume (in $\mu\text{l g}^{-1}$) of interstitial extracellular fluid in the testes of (○) control rats (group C), (□) rats treated with a Zoladex implant and 12 daily injections of Flutamide from day -21 to day -10 (65–76 days of age; group ZF), (●) rats in which the testes were heated by immersion in a waterbath at 43°C for 30 min on day 0 (86 days of age; group H), and (■) rats exposed to both treatments (group HZF). H35 ($P < 0.001$), H70 ($P < 0.05$) and H105 ($P < 0.01$) were significantly greater than corresponding C values; HZF35 was significantly greater than ZF35 ($P < 0.05$); ZF0 was significantly greater than ZF70 and ZF105 ($P < 0.01$).

decrease in the number of spermatozoa in both the testis and the epididymis at day 35, with partial recovery at day 70, and further recovery in the number of spermatozoa in the epididymis at day 105, although at this time, the values were still lower than those in controls. However, there was no further improvement in the number of spermatozoa in the testis between day 70 and day 105, and at both times the values were lower than those in controls (Fig. 3).

Fluid volumes in the testis

The volume of interstitial extracellular fluid in the testis, expressed per unit weight of tissue, was increased in the hormone-treated rats by day 0, but then decreased to control values by day 35 and thereafter. Heating the testes also caused an increase in interstitial extracellular fluid, which returned to control values in HZF rats at days 70 and 105, but was still high at day 70 and higher again at day 105 in rats in group H (Fig. 4).

There were no consistent effects of heat or hormone treatment on the volume of tubular fluid, which remained at about $250 \mu\text{l g}^{-1}$ throughout the experiment (data not shown), as in the previous experiment (Setchell *et al.*, 2001).

Histology of the testis

As reported previously (Setchell *et al.*, 2001), seminiferous tubule diameter was significantly reduced by Zoladex-

Fig. 5. Diameter (in μm) of the seminiferous tubules of (○) control rats (group C), (□) rats treated with a Zoladex implant and 12 daily injections of Flutamide from day -21 to day -10 (65–76 days of age; group ZF), (●) rats in which the testes were heated by immersion in a waterbath at 43°C for 30 min on day 0 (86 days of age; group H), and (■) rats exposed to both treatments (group HZF). H35 ($P < 0.001$), H70 and H105 ($P < 0.05$) were significantly less than corresponding C values; H35 was significantly less than HZF35 ($P < 0.05$), H70 ($P < 0.001$) and H105 ($P < 0.01$); HZF35 was significantly less than ZF35 ($P < 0.05$); ZF0 was significantly less than all C values and HZF and other ZF values ($P < 0.05$).

flutamide treatment. In the present study, the diameter of the seminiferous tubules was reduced to about 60% of the normal diameter between day -21 and day 0. The diameter subsequently returned to normal by day 35 and remained normal for the rest of the experiment. The diameter of the tubules in hormone-treated animals in which the testes were heated on day 0 recovered more slowly, and at day 70 after heating some, but not all, animals still showed decreased tubular diameters. In the non-hormone-treated rats in which the testes were heated, there was also a variable response in tubular diameter but the mean was significantly lower than that in control rats at all times examined, so that even at day 105 after heating, the mean seminiferous tubular diameter was still reduced (Fig. 5).

Histological examination of the testes of hormone-treated rats at the time of heating showed that spermatogenesis was severely impaired in all rats examined: differentiation through and beyond the meiotic divisions had occurred in only one rat to a very limited extent. Most tubules appeared to contain only Sertoli cells, spermatogonia and a few early spermatocytes. In one rat a small number of pachytene spermatocytes and early spermatids were present in a few tubules, but none beyond stage 9. As spermatogenesis was disrupted so severely, it was impossible to distinguish spermatogenic stages (Fig. 6). These effects appear to be greater than those reported by

Kangasmiemi *et al.* (1995), using a different strain of rats. There was also a significant increase at day 35 after heating in the proportion of tubule cross-sections showing abnormal histology in the four rats pretreated with Zoladex and flutamide (group HZF) compared with that in the hormone-treated rats that were not exposed to heat (group ZF). In one animal there were no elongated spermatids, and in another there were only very few tubules with cells more advanced than the round spermatid stage. In the other two rats in this group, there were some elongated spermatids, but there was significant disruption of meiosis and spermiogenesis at the round spermatid stage. In the hormone-treated rats that had not been exposed to heat (group ZF), spermatogenesis was normal at day 35, that is, 56 days after the start of hormone treatment. There were no differences in the histology of testes from rats in groups HZF and ZF at days 70 or 105 (Table 2).

The changes in the histology of the testes of non-hormone-treated rats in which the testes were heated were very similar to those reported by Setchell *et al.* (2001), with complete disruption of spermatogenesis at day 35 after heating. By day 70, there was partial recovery in the percentage of normal tubules in group H, but the number had decreased again significantly by day 105.

Discussion

The most important finding in the present study is that, even when the most susceptible cells of the testis (pachytene spermatocytes and young spermatids: Lue *et al.*, 1999; for earlier references see Setchell, 1998) are absent or severely reduced in number, other cells in the testis are affected by heat. Testis mass and the number of spermatozoa in the testis and epididymis were lower in animals in which the testes were heated 35 days earlier while spermatogenesis was maximally suppressed, than in animals receiving only the hormone pre-treatment with a GnRH agonist and an anti-androgen to suppress spermatogenesis. Cells that had become homogenization-resistant spermatids in the testis at day 35 would have been pre-leptotene spermatocytes at the time of heating, assuming a duration of 13 days for each spermatogenic cycle in rats and the requirement of three cycles for B spermatogonia to develop to late spermatids (see Russell *et al.*, 1990), the nuclei of which are resistant to homogenization (Robb *et al.*, 1976). Cells that had become homogenization-resistant spermatids at day 70 or day 105 would have been spermatogonia at the time of heating. It is not clear from the present or previous results whether the spermatogonia are susceptible to heat effects, or whether their subsequent development is inhibited by an effect of the heat on the Sertoli cells. The cells usually regarded as being most susceptible to heat would have left the testis between day 15 and day 32 after heating, values that correspond closely to the times at which minimum sperm counts were observed in rete testis fluid collected from heat-exposed rat testes (Setchell and Waites, 1972).

However, although either spermatogonia or their

Fig. 6. (a) Seminiferous tubules at the time of heating (day 0) from a rat treated with a GnRH agonist (Zoladex) 21 days earlier (day -21) and the anti-androgen flutamide between day -21 and day -10. Note that most germ cells are missing and large lipid droplets are present. (b) Seminiferous tubules of a control (no hormone treatment) rat at day 35 after heating of the testes, showing disruption of spermatocytogenesis: no meiotic or post-meiotic cells are present. (c) Two seminiferous tubules of a rat treated with Zoladex on day -21 and flutamide from day -21 to day -10, in which the testes were heated on day 0 and sampled on day 35. Note the severe disruption of spermatogenesis in the lower tubule, although disruption is not as severe as in the non-hormone-treated rat exposed to heat, whereas in the top tubule, the only missing cells are elongated step 18 spermatids. (d) Parts of a tubule from a rat treated with Zoladex on day -21 and flutamide between day -21 and day -10 and sampled on day 35. Spermatogenesis is complete in this tubule at late stage VIII with only a few step 19 spermatids remaining attached to the Sertoli cells. All light micrographs are from semi-thin sections stained with toluidine blue. Scale bar represents 50 μ m.

development are affected by heat, the effect appears to be smaller in animals in which spermatogenesis is suppressed at the time of heating than in animals subjected to the same heat treatment but with normal spermatogenesis at the time of heating. Animals with normal spermatogenesis at the

time of heating show lower sperm counts in the testis at day 35 than do hormone-treated rats in which the testes are heated, and this difference persists at least until day 70 after heating. The discrepancy between these changes and those seen in numbers of spermatozoa in the epididymis, which

Table 2. Numbers of seminiferous tubular cross-sections examined and percentages that appeared qualitatively normal in control rats (C), in rats treated with a Zoladex implant and 12 daily injections of Flutamide from day -21 to day -10 (ZF), in rats in which the testes were heated by immersion in a waterbath at 43°C for 30 min (H) on day 0, and in rats exposed to both treatments (HZF)

Time after heating (days)	C		H		HZF		ZF	
	Total number of tubules (per rat)	Normal tubules (%)	Total number of tubules (per rat)	Normal tubules (%)	Total number of tubules (per rat)	Normal tubules (%)	Total number of tubules (per rat)	Normal tubules (%)
0							60 (13–34)	0
35	38 (8–18, 3)	100	57 (10–20, 4)	0 ^c	70 (12–25, 4)	87 ^{ad}	50 (11–20, 3)	100
70	32 (10–11, 3)	100	39 (12–27, 4)	87 ^b	53 (9–22, 4)	100	61 (19–21, 3)	95
105	40 (10–21, 3)	100	76 (10–21, 5)	66 ^{ce}	70 (9–21, 5)	96 ^a	52 (12–38, 3)	100

All available tubule cross-sections from all rats were assessed; the number of assessable tubules was determined by the size and orientation of the blocks. Ranges of the numbers of assessable tubules for each rat and the number of rats in each group are given in parentheses.

^a $P < 0.001$ greater than group H.

^b $P < 0.05$, ^c $P < 0.001$ different from group C.

^d $P < 0.05$ different from group ZF.

^e $P < 0.05$ less than group H at day 70.

are actually higher in the untreated rats at day 35, but lower at both day 70 and day 105, is presumably a result of the delay between the appearance of homogenization-resistant spermatid nuclei in the testis and their passage to the tail of the epididymis, where more than half of the epididymal spermatozoa are found (Robb *et al.*, 1976). It is apparent that, in the testes of non-hormone-treated rats in which the testes are heated, the recovery of the less developed cells is impeded, possibly because the testosterone concentrations are higher than in the rats treated with GnRH agonist–anti-androgen, as indicated by the lower seminal vesicle masses in the hormone-treated animals. Such an inhibitory effect of high testosterone concentrations in the testis on repopulation of the spermatogenic epithelium was reported after irradiation or cytotoxic drug treatment (Meistrich and Kangasniemi, 1997; Shetty *et al.*, 2000).

The failure of the testis mass, seminiferous tubule diameter and sperm count to return to control values at day 105 after heating confirms the results of the study by Setchell *et al.* (2001). Although the duration of the present study was not sufficient to reveal the second decline in testis mass and number of spermatozoa seen in the study of Setchell *et al.* (2001), it provides further evidence for an effect of heat on the less developed germ cells in the testis or on their subsequent development. Further evidence for such a long-term effect was reported by Rockett *et al.* (2001), who showed that testis mass in mice was lower than that in controls at day 68 after heating, the longest period considered in the study.

The effects of the GnRH agonist and anti-androgen treatment on body weight gain are also in accord with earlier results (Setchell *et al.*, 2001), as are the results from determination of the volumes of interstitial extracellular and tubular fluids, except that the increase in interstitial extracellular fluid at day 35 after heating was much greater than that seen in the first experiment, for no apparent reason. The increased volume of interstitial extracellular fluid seen on

day 0 (21 days after the start of GnRH agonist–anti-androgen treatment) was more obvious than the small but significant increase seen at day 35 in the previous experiment; however, the shorter time interval between treatment and sampling could explain this difference. The reason for the larger volume of fluid outside the tubules when spermatogenesis is suppressed, whether by heat or by hormone treatment, is still unclear, but may be a result of the decreased volume of the seminiferous tubules.

In conclusion, the present study provides further evidence that, in addition to the known effects of short-term local heating on young spermatids and pachytene spermatocytes, germ cells at earlier stages of development are also affected by heat.

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References

- Hild SA, Meistrich ML, Blye RP and Reel JR (2001) Lupron depot prevention of antispermatogetic/antifertility activity of the indenopyridine, CDB-4022, in the rat *Biology of Reproduction* **65** 165–172
- Kangasniemi M, Wilson G, Huhtaniemi I and Meistrich ML (1995) Protection against procarbazine-induced testicular damage by GnRH-agonist and antiandrogen treatment in the rat *Endocrinology* **136** 3677–3680
- Lue YH, Sinha Hikim AP, Swerdloff RS, Im P, Khay ST, Bui T, Leung A and Wang C (1999) Single exposure to heat induces stage-specific germ cell apoptosis in rats: role of intratesticular testosterone on stage specificity *Endocrinology* **141** 1709–1717
- Meistrich ML and Kangasniemi M (1997) Hormone treatment after irradiation stimulates recovery of rat spermatogenesis from surviving spermatogonia *Journal of Andrology* **18** 80–87
- Robb GW, Amann RP and Killian GJ (1976) Daily sperm production and epididymal sperm reserves of pubertal and adult rats *Journal of Reproduction and Fertility* **54** 103–107
- Rockett JC, Mapp FL, Garges JB, Luft JC, Chisato C and Dix DJ (2001)

- Effects of hyperthermia on spermatogenesis, apoptosis, gene expression and fertility in male mice *Biology of Reproduction* **65** 229–239
- Russell LD, Ettlin RA, Sinha Hikim AP and Clegg ED** (1990) *Histological and Histopathological Evaluation of the Testis* Cache River Press, Clearwater, FL
- Setchell BP** (1998) The Parkes lecture: heat and the testis *Journal of Reproduction and Fertility* **114** 179–194
- Setchell BP and Waites GMH** (1972) The effects of local heating on the flow and composition of rete testis fluid in the rat, with some observations on the effects of age and unilateral castration *Journal of Reproduction and Fertility* **30** 225–233
- Setchell BP, Plöen L and Ritzen EM** (1999) Effect of heating on rat testes with suppressed spermatogenesis *Journal of Reproduction and Fertility Abstract Series* **23** 30
- Setchell BP, Plöen L and Ritzen EM** (2001) Reduction of long-term effects of local heating of the testis by treatment of rats with GnRH agonist and an anti-androgen *Reproduction* **122** 255–263
- Shetty G, Wilson G, Huhtaniemi I, Shuttlesworth GA, Reissmann T and Meistrich ML** (2000) Gonadotrophin-releasing hormone analogs stimulate and testosterone inhibits the recovery of spermatogenesis in irradiated rats *Endocrinology* **141** 1735–1745
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