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Experimentally Induced Testicular Alterations in Boars: Hormonal Changes in Mature and Peripubertal Boars

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Malmgren, L.: Experimentally induced testicular alterations in boars: Hormonal changes in mature and peripubertal boars. Acta scand. vet. 1990, 31, 97-107. – Eleven sexually mature boars and 10 peripubertal boars were used to study the effects of elevated testicular temperature on plasma hormonal levels. The scrotum of the boars was covered with a textile-aluminium foil insulation device for 100 h. Insulation of the scrotum in the peripubertal boars took place at an age of 100 days.

Blood samples were drawn 3 times daily for 12 days in the mature boars, starting 3 days before scrotal insulation. In the peripubertal boars, blood sampling was performed once a day for 11 days, starting the first day of scrotal insulation.

During scrotal insulation, the plasma levels of testosterone, oestradiol-17 β and oestrone sulphate decreased continuously in the mature boars. After removal of the scrotal insulation device there was a continuously increase, back to normal levels of oestradiol-17 β and oestrone sulphate. The plasma levels of testosterone showed an immediate rise of brief duration after removal of the device in 5 of the boars, while in the other 6 boars the rise in testosterone levels came 4 days after removal and lasted for 3 days.

In the peripubertal boars, there were no significant differences in the hormone levels between the experimental and control animals during and after scrotal insulation. However, the decrease in testosterone concentration over time, during scrotal insulation, was significant within the experimental group.

Boar; mature; peripubertal; testosterone; oestradiol-17 β ; oestrone sulphate.

Introduction

Various environmental factors are known to influence gonadal function. Prolonged stress in man was found to have a suppressive effect on plasma testosterone concentrations (*Aakvaag et al.* 1978). Decreased testosterone concentrations were also seen in boars (*Larsson et al.* 1983) and in rams (*Gomes* 1971) after heat stress.

The testes of boars and stallions are very active in producing oestrogens, and particularly oestrogen sulphate is present in high concentrations in the peripheral plasma in these species. Oestrogens are formed in testicular tissue by conversion of testosterone (*Setchell* 1978).

Experimentally induced cryptorchidism in rats (*Berg & Damber* 1978) caused a great reduction in testosterone levels in the abdominal testicle. *Mazzarri et al.* (1968) and *Holst* (1949) showed that local heating of the scrotum caused alterations in the seminiferous epithelium, but they did not study the hormonal patterns. We have developed a reliable method for scrotal insulation which elevates the scrotal surface temperature 3-5°C. Boar testis exposed to such a high surface temperature develop degenerative changes

in the seminiferous epithelium (*Malmgren & Larsson* 1989). These changes are accompanied by altered sperm morphology for about 6 weeks (*Malmgren* 1989) in mature boars. In peripubertal boars, maturation of the testicular function was delayed if scrotal insulation was performed around the time of onset of spermatogenesis, i.e. at an age of 100 days (*Malmgren* 1989).

Florcruz & Lapwood (1978), *Allrich et al.* (1982) and *Ford* (1983) studied the endocrine changes occurring during sexual maturation in boars under normal conditions. There seems to be differences in the testosterone secretion patterns as various studies have indicated a presence (*Claus & Gimenez* 1977, *Kattesh et al.* 1982) or absence (*Brock & Wetteman* 1976, *Lapwood & Florcruz* 1978, *Edqvist et al.* 1980) of a diurnal testosterone secretion pattern.

To our knowledge no study has been performed on the effect of elevated scrotal temperature after scrotal insulation in peripubertal or mature boars on hormone secretion. Although it is obvious and well known that increased scrotal temperature causes degeneration of the seminiferous epithelium, the underlying mechanisms are still poorly understood. Since hormonal changes have been found to occur after exposing boars to heat stress it is important to study the endocrine effects of local scrotal heating, avoiding exposure to any external stress on the animals. The purpose of this study was to describe the temporal changes in peripheral plasma concentrations of testosterone, oestradiol-17 β and oestrone sulphate during and after scrotal insulation in mature and peripubertal boars.

Material and methods

Animals

Eleven sexually mature Swedish Landrace experimental boars (8-12 months of age) and 10 peripubertal (6 experimental and 4 control animals) crossbred (Swedish Landrace/Swedish Yorkshire) boars were used in the study. Seven of the mature boars and 8 of the peripubertal boars were also used for studies of the semen and sperm morphology (*Malmgren* 1989). The animals were kept in a barn at an ambient temperature of approximately 18°C. The mature boars were kept in individual pens. The peripubertal boars, which originated from 3 different litters, were penned together. All boars were fed a commercial feed used for finishing pigs in Sweden. Scrotal insulation was performed as previously described (*Malmgren & Larsson* 1989).

Blood sampling

In the mature boars permanent vein catheters were placed in the jugular vein and passed subcutaneously to the animals back (*Rodriguez & Kunavongkrit* 1983). Blood samples were drawn via the catheters, without restraining the animals, 3 times daily (9 a.m., noon, 3 p.m.) for 12 days, starting 3 days before scrotal insulation. In the peripubertal boars blood sampling was performed once a day for 11 days, starting on the first day of scrotal insulation when the boars were 100 days of age. The blood samples were taken from the jugular vein, using heparinized vacutainer tubes, while the boars were restrained. The blood samples were collected in heparinized tubes, immediately centrifuged and the plasma was harvested and stored at -20°C until assay.

Hormone assays

All the hormone analyses were carried out by radioimmunoassay, at the Department of Clinical Chemistry, Swedish University of Agricultural Sciences.

Antiserum to testosterone was raised against testosterone-3-(O-carboxymethyl)oxime-b-

ovine serum albumin. The crossreactivity of this antiserum with other related steroids indicated significant crossreaction with 5α-dihydrotestosterone 61% and androstenedione 7% (*Oltner et al.* 1979). The inter-assay coefficient of variation was 7% at an average concentration of 18.9 nmol/l).

Oestradiol-17 β was determined using an antiserum against 6-ketooestradiol-17 β-6-(O-carboxy-ethyl)oxime-bovine serum albumin which crossreacted 11% with oestrone (*Boilert et al.* 1973, *Lindberg et al.* 1974). The inter-assay coefficient of variation was 16% and 7% at average concentrations of 25.7 and 112.2 pmol/l, respectively.

Oestrone sulphate was determined using an antiserum against oestrone glucosiduronate-bovine thyreoglobulin (*Wright et al.* 1978). The antiserum crossreacted with oestrone 62.5%, 2-hydroxoestrone 24.3%, oestradiol-17 β 15.9%, oestrone-glucosiduronate 10.7% and oestradiol-3-glucosiduronate 2.6% (*Wright et al.* 1978). The inter-assay coefficient of variation was 8% and 10% at average concentrations of 9.6 and 14.1 nmol/l, respectively.

All hormone concentrations reported represent the mean of duplicate determinations.

Statistical analysis

The checking and handling of the data as well as the statistical analysis were carried out using the Statistical Analysis System (*SAS Institute Inc.* 1985).

For the mature boars, the hormone levels were analysed using analysis of variance, according to a model including the effects of boar and sampling day.

For the peripubertal boars, the hormone levels were analysed for each sampling day, using analysis of variance, according to a model including the effects of group and boar within group.

The samples collected during scrotal insulation (days 1 to 5) were analysed, for each group of boars, according to a model including the effects of boar and regression on day identity. In the above model, the effect of boar was regarded as random. Levels of significance are expressed conventionally: $p<0.05^{*}$, $p<0.01^{**}$, $p<0.001^{***}$.

Results

Mature boars

The average levels of testosterone decreased continuously during scrotal insulation from 14.5 nmol/l to 7.5 nmol/l. The testosterone levels during scrotal insulation differed significantly from the preexperiment concentration (Fig. 1a). Also the decrease over time during scrotal insulation was significant (regression coefficient = -1.07*** nmol/l/day). After removal of the scrotal insulation device the changes in the hormonal concentrations showed 2 different patterns among the boars. Five of the boars got an immediate and brief rise in testosterone on the day following removal of the scrotal device. (Fig. 1b). The rise in testosterone concentrations in the other 6 boars came 4 days after removal of the device and lasted for 3 days (Fig. 1b). It should be mentioned that the latter group of boars had higher average testosterone levels during the whole scrotal insulation period than the former group.

Concentrations of oestradiol-17β and oestrone sulphate are shown in Figs. 2 and 3. Both hormones decreased continuously during scrotal insulation, and the concentrations were significantly lower than the pretreatment level during most days of scrotal insulation. Oestradiol-17β decreased from 236 pmol/l to 173 pmol/l and oestrone sulphate decreased from 43 nmol/l to 17 nmol/l, respectively, from the day before the start of treatment to day 5 of treatment. After removal of the insulation device concentrations of both hormones began to in-

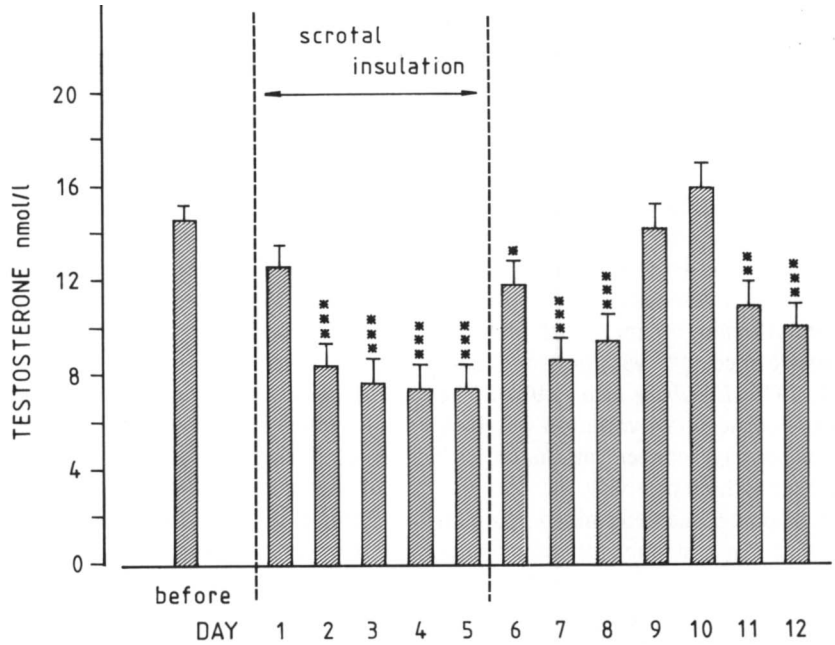

Figure 1a. Daily least squares means levels of testosterone before, during and after scrotal insulation in the mature boars. The »before« value refers to least squares means the 3 days (3 samples a day) before scrotal insulation. * = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$, compared with »before« value.

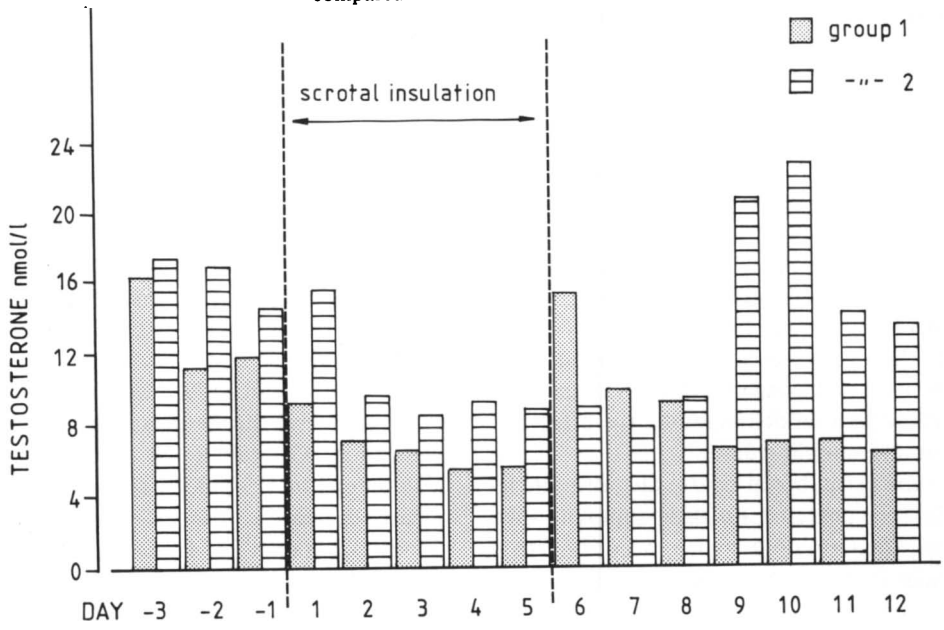

Figure 1b. Daily means in the 2 groups of mature boars with different hormonal patterns after scrotal insulation. Group 1 = 5 boars. Group 2 = 6 boars.

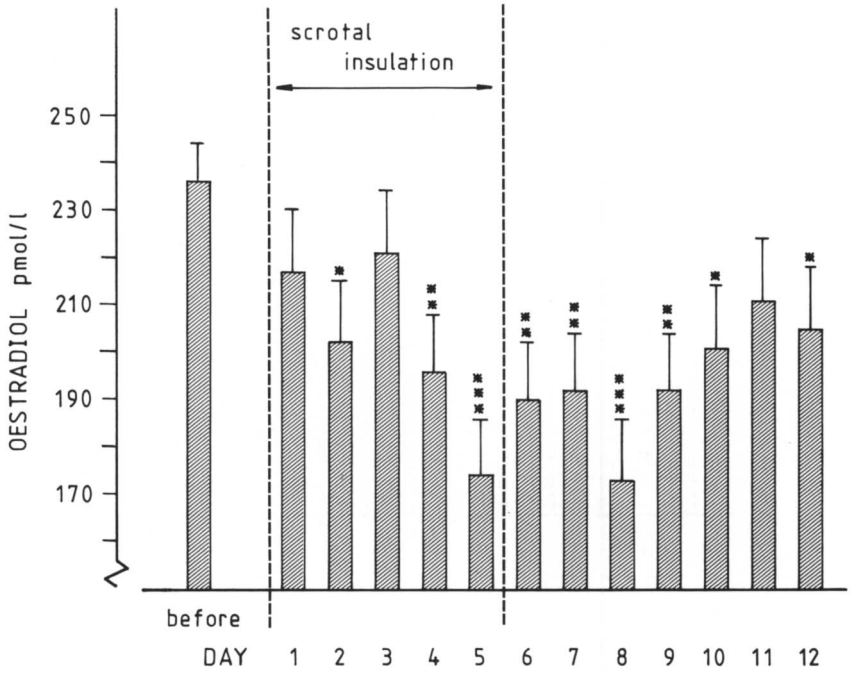

Figure 2. Daily least squares means levels of oestradiol-17β before, during and after scrotal insulation in the mature boars. The »before« value refers to least squares means the 3 days (3 samples a day) before scrotal insulation. * = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$, compared with »before« value.

crease and a day 6 after removal, no significant differences, compared with pretreatment levels, were seen. The levels of oestradiol-17β and oestronesulphate were then 211 pmol/l and 40 nmol/l, respectively.

Peripubertal boars

The daily least-squares means for testosterone, oestradiol-17β and oestrone sulphate in the peripubertal boars during and after scrotal insulation is given in Figs. 4-6.

Testosterone concentrations in the experimental group decreased during scrotal insulation, from 8.8 nmol/l to 4.6 nmol/l, but the decrease was not significant compared with the control group. However, the decrease over time during scrotal insulation was significant within the experimental group (regression coefficient = -1.33* nmol/l/day). After removal of the scrotal insulation device testosterone was almost similar in both groups (ranging from 4.0 nmol/l to 7.2 nmol/l) (Fig. 4).

There were no significant differences in oestradiol-17β and oestrone sulphate between the control and experimental animals. Oestradiol-17β in the experimental group ranged from 53.3 pmol/l to 95.5 pmol/l and in the control group from 75 pmol/l to 109.0 pmol/l (Fig. 5). Oestrone sulphate ranged from 19.5 nmol/l to 45.0 nmol/l in the experimental group and from 19.0 nmol/l to 36.0 nmol/l in the control group (Fig. 6).

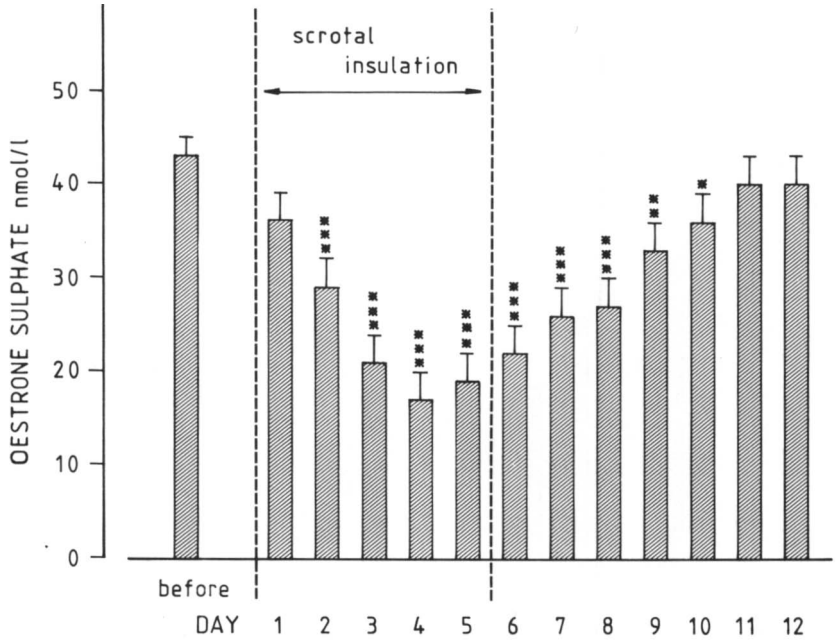

Figure 3. Daily least squares means of oestrone sulphate before, during and after scrotal insulation in the mature boars. The »before« value refers to least squares means the 3 days (3 samples a day) before scrotal insulation. * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$, compared with »before« value.

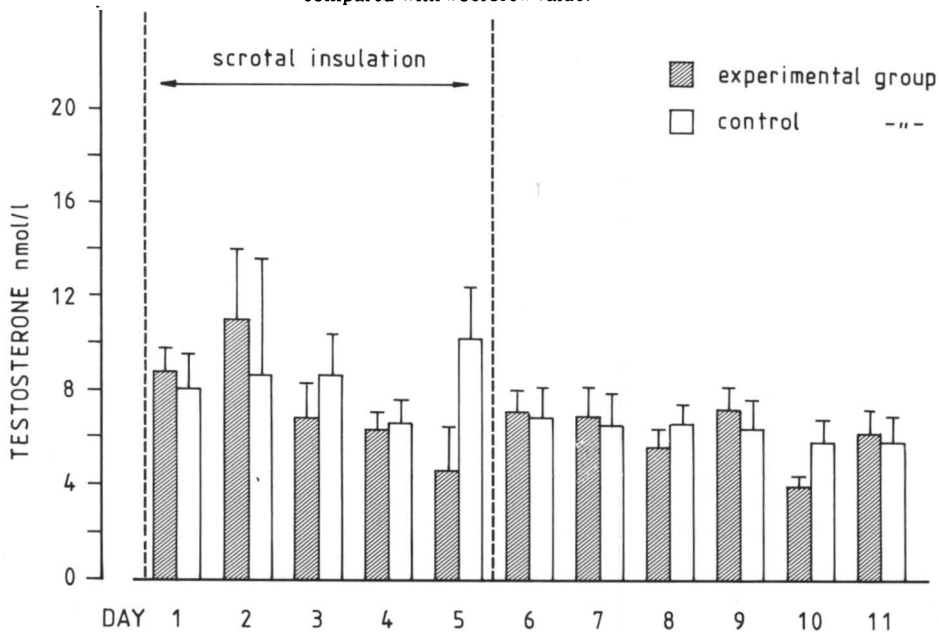

Figure 4. Daily least squares means levels of testosterone for experimental (scrotal insulation) and control peripubertal boars. No significant differences were seen between the groups. A significant decrease over time, during scrotal insulation, was seen within the experimental group.

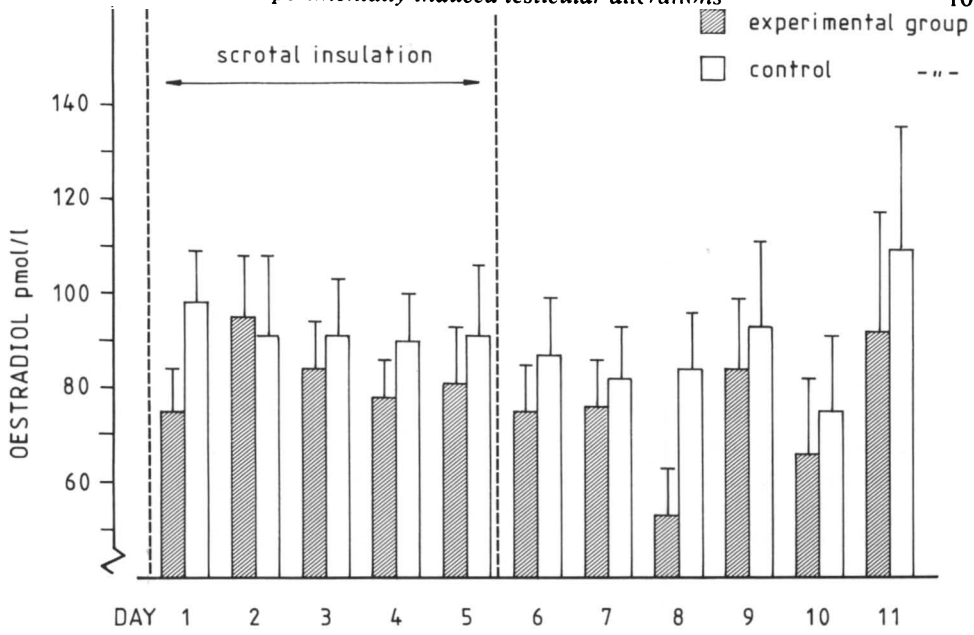

Figure 5. Daily least squares means levels of oestradiol-17β for experimental (scrotal insulation) and control peripubertal boars. No significant differences were seen between the groups.

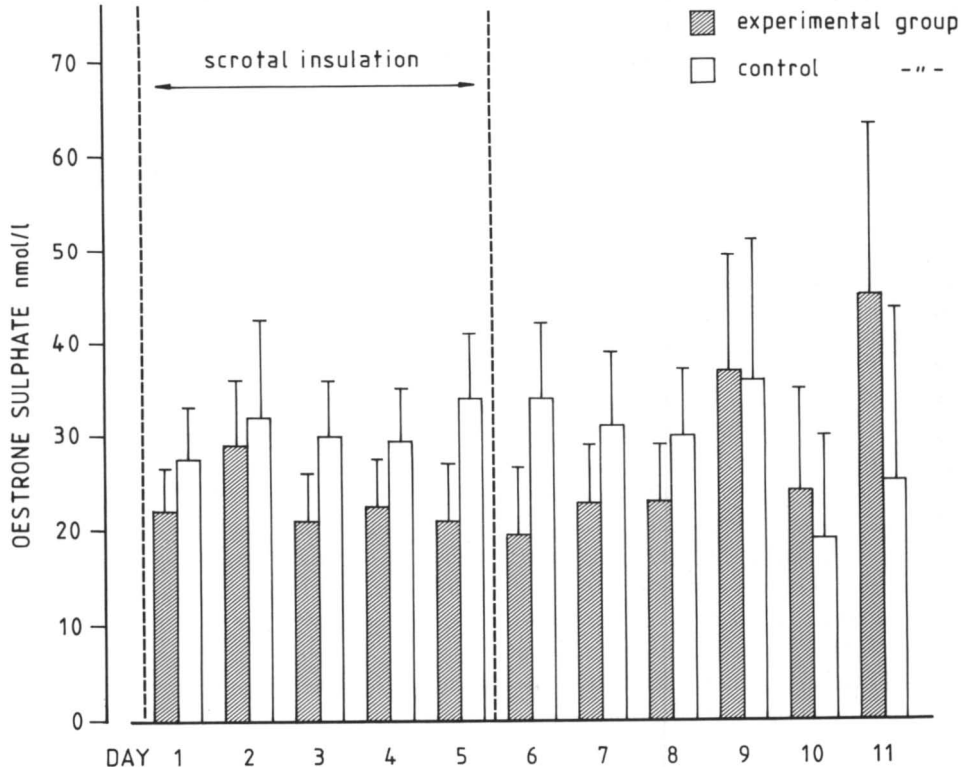

Figure 6. Daily least squares means of oestrone sulphate for the experimental (scrotal insulation) and the control groups. No significant differences were seen between the groups.

Discussion

The decrease in plasma testosterone concentrations during scrotal insulation in the mature boars is in agreement with previous experiments with boars exposed to heat stress (*Wetteman & Desjardin* 1979, *Larsson et al.* 1983). They are also in accordance with findings in rams after stress exposure (*Gomes et al.* 1971) or challenge with endotoxin (*Wallgren et al.* 1989). A marked suppressive effect on plasma testosterone levels was seen in men exposed to prolonged physical and psychological stress, (*Aakvaag et al.* 1978). In boars treated with ACTH for 5 days reduced testosterone concentrations were observed (*Liptrap & Raeside* 1975).

In the present study using only insulation of the scrotum and in which the boars were maintained in a familiar environment, one may assume that the stress factor on the animals was minimal. Thus, plasma cortisol levels may remain unaffected and should not cause a reduction in the plasma testosterone secretion. It seems more likely that the changes in testosterone secretion are caused by a direct effect of the higher temperature on the testis. This was previously found by *Berg & Damber* (1978), *Damber et al.* (1978) in rats, and *Skinner & Rowson* (1968) in lamb and calves, where lowered testosterone concentrations in experimentally induced cryptorchidism indicated impaired Leydig cell function.

The elevated testosterone found after termination of the scrotal insulation is most likely intended to compensate for the lowered levels observed during scrotal insulation. The pattern, including the variations among boars, is very similar to the results after heat stress exposure (*Larsson et al.* 1983). It is interesting to note that the testosterone elevation occurred later and lasted longer in boars which had comparatively higher testosterone levels during scrotal insulation. This may indicate an individual decreased sensitivity to increased temperature and also less need for compensatory testosterone production.

The decrease in oestradiol-17β and oestrone sulphate concentrations is likely to be due to the lower testosterone production, since oestrogens are synthesized from androgens (*Dorrington et al.* 1978) in the testicles. If the androgen concentration, e.g. testosterone and androstenedione, is lowered the conversion rate to oestrogens may decrease. *Reaside & Renaud* (1983), suggested that oestrogen production in the boars is due mainly to the steroidogenic capacity of the Leydig cells. Another explanation could be impaired Sertoli cell function, caused by the elevated temperature, since *Dorrington et al.* (1978) suggested that the synthesis of oestrogens takes place in the Sertoli cells influenced by FSH. *Hagenäs & Ritzen* (1976) showed that after experimentally induced cryptorchidism in the rat the production of seminiferous fluid decreases indicating that the Sertoli cell secretory function was impaired by the elevated temperature. However, under the experimental conditions used it can only be stated that elevated scrotal temperature in the mature boars caused lowered peripheral concentrations of both testosterone and oestrogens.

Age and maturity seemed to influence the endocrine response. There were no significant differences between the peripubertal experimental and control boars when comparison was made within days. There was, however, a significant decrease in plasma testosterone levels, during scrotal insulation, over time within the experimental group. Plasma oestrogen levels showed only small differences between the experimental and control animals. The explanation could be that the samples were taken at around 100 days of age. At this age spermatogenesis begins (*Florcruz & Lapwood* 1978) and the en-

docrine system involving the testes is very active. Oestrogen and testosterone concentrations increase continuously as the pubertal development progresses (*Florcruz & Lapwood* 1978, *Allrich et al.* 1982). *Allrich et al.* (1982) suggested that there is a higher sensitivity of the hypothalamus/pituitary to the negative steroid feedback controlling LH at this age. It is further suggested that the metabolic clearance rate of testosterone may be lower during the peripubertal period.

Another and equally plausible explanation of the minor endocrine response among the peripubertal boars may be the scheme and procedure used for blood sampling. The one sample per day procedure with restraining of the animals may have masked substantial endocrine alterations. This short stress situation could induce high cortisol levels and an increase in testosterone concentrations (*Liptrap & Raeside* 1975, *Juniewicz & Johnson* 1981). It was originally planned that the peripubertal boars should be sampled in exactly the same way as the older boars. Preliminary attempts, however, showed that the problem with infected permanent vein catheters was so great that this method had to be abandoned for the young boars. Similar problems never occurred with the mature boars. In an additional experiment boars were treated with testosterone propionate for 4 weeks (*Malmgren* 1988). The dosage levels were chosen so that peripheral plasma testosterone levels were maintained within the ranges found in the present study before scrotal insulation. This plasma level probably gives a negative feed back to the hypothalamus/pituitary glands, which leads to a decrease in LH release. It may be assumed that the boars treated in this way had substantially lowered intratesticular testosterone levels (*Huang & Nieschlag* 1984). Yet, their semen production and sperm morphology remained unaffected. The reduced testosterone production during heat stress or scrotal insulation is thus not likely to be the direct cause of the changes found in the seminiferous epithelium and sperm morphology. It seems more likely that the endocrine changes, as well as the changes in the seminiferous epithelium, both result from the increased testicular temperature.

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Sammanfattning

Experimentellt framkallad testikeldegeneration hos galt: hormonella förändringar hos könsmogna och pubertala galtar.

Elva könsmogna och 10 peripubertala galtar användes för att studera effekten av förhöjd testikeltemperatur på hormonnivåerna i blodet. Skrotum täcktes med en värmeisolerande påse i 100 tim. På de unga galtarna skedde skrotumisoleringen vid 100 dagars ålder. Blodprov togs från de könsmogna galtarna 3 ggr/dagl. i 12 dagar, med början 3 dagar innan skrotumisoleringen. Från de peripubertala galtarna togs blodprov 1 gång dagl. i 11 dagar, med början samma dag som skrotumisoleringen startade.

Under skrotumisoleringen av de könsmogna galtarna sjönk perifera plasmanivåerna av testosteron, östradiol-17β och östron-sulfat. När isoleringen togs bort ökade nivåerna av östradiol-17β och östronsulfat succesivt tillbaka till ursprungvärdena. Plasmanivåerna av testosteron uppvisade en snabb ökning med kort duration efter avtagandet av skrotumisoleringen hos 5 av galtarna, medan hos de andra 6 kom ökningen 4 dagar senare och varade i 3 dagar.

Hos de peripubertala galtarna var det ingen signifikant skillnad, beträffande hormonnivåerna, mellan experiment och kontrolldjuren under och efter skrotumisoleringen. Däremot var sänkningen av testosteronnivåerna över tiden signifikant hos experimentgruppen under skrotumisoleringen.

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