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From the Departments of Obstetrics and Gynaecology and Clinical Chemistry, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden.

ENDOCRINE EFFECTS OF HEAT STRESS IN BOARS

By

K. Larsson, S. Einarsson, K. Lundström and *J. Hakkarainen*

LARSSON, K., S. EINARSSON, K. LUNDSTRÖM and J. HAKKARAINEN: *Endocrine effects of heat stress in boars.* Acta vet. scand. 1983, *24*, 305—314. — The purpose of this study was to describe the temporal changes in peripheral plasma levels of testosterone and cortisol in boars during and after heat stress. A total of 8 boars were utilized, 4 of them were exposed to 35°C, for 100 h in a climatic room, and 4 served as controls and were kept at 20°C for 100 h in the climatic room.

Blood samples were obtained via permanent vein catheters 3 times daily from 5 days before heat stress until 20 days after termination of heat stress. Testestorone levels were determined by radioimmunoassay and cortisol by a competitive protein binding technique.

For both hormones the pre-exposure levels were similar in both groups of boars. The control boars had significantly higher testosterone levels, while being in the climatic room, than during any other period. The experimental boars had slightly increased testosterone levels during the first day of heat stress and thereafter continuously decreased levels. In the control boars the testosterone levels returned to pre-exposure levels immediately after removal from the climatic room, whereas in the experimental boars the testosterone levels were dramatically increased during the first 5 days after exposure. The differences in cortisol levels, between the 2 groups of boars were restricted to the period spent in the climatic room. During this period the experimental boars had significantly higher cortisol levels.

boars; heat stress; testosterone; cortisol.

Testicular function in boars is influenced by a number of external factors such as elevated temperature (*McNitt & First* 1970, *Wetterman et al.* 1976, 1977), confinement (*Andresen* 1975) and social environment (*Andresen* 1976). Local heating of the scrotum causes degeneration of the seminiferous epithelium in the pig (*Holst* 1949) as well as in other species.

Heat stress also affects mating behaviour (*Winfield et al.* 1981) such that heat stressed boars display less courting activity. Decreased peripheral plasma testosterone levels were found in boars after 1 week of heat stress (*Wetteman & Desjardin* 1979). The underlying mechanisms are not fully understood. However, a relationship seems to exist between adrenal corticoid secretion and testicular steroid secretion. Thus *Liptrap & Raeside* (1978) found a sharp increase in plasma corticosteroid levels together with increased testosterone levels in boars, after copulation or confrontation with aggressive boars. Similar results were obtained after ACTH treatment in boars (*Juniewicz & Johnson* 1981) and after brief stress in bulls (*Welsh & Johnson* 1981).

The influence of duration of heat stress and the longterm effects of stress have been studied on a limited scale only. *Einarsson & Larsson* (1980) found dramatically increased peripheral plasma testosterone levels in boars after termination of 100 h of exposure to elevated ambient temperature. The testosterone levels remained high for at least 5 days, but when the stress period was restricted to 24 h only minor endocrinological changes were observed.

The purpose of the present study was to describe the temporal changes in peripheral plasma levels of testosterone and cortisol during and after heat stress.

MATERIAL AND METHODS

Animals

Eight sexually mature Swedish Landrace boars were utilized. All boars originated from the same herd and they were brought to the clinic at approximately 7 months of age. At the clinic they were kept in individual pens at barn temperature (approx. 18°C) and fed a standard ration of grain mix and concentrate. Semen was collected twice weekly from all boars for morphological examination. All boars produced at least 8 ejaculates with normal sperm morphology, according to the standards of our laboratory, before exposure to heat stress.

Some 4—6 weeks after their arrival, permanent vein catheters were inserted into the brachial vein and passed subcutaneously to the animals back, according to the method of *Karlbom et al.* as described by *Shille et al.* (1979). Blood sampling via the catheters was then performed, without restraining the animals,

3 times each day (9 a.m., noon, 3 p.m.) throughout the experimental period. The blood samples were collected into heparinized tubes, centrifuged immediately and the plasma was harvested and stored at below —20°C until assayed. Testosterone levels were determined by radioimmunoassay (*Oltner et al.* 1979) and cortisol by a competitive protein binding technique (*Snochowski et al.* 1981). Peripheral plasma hormone levels were monitored from 5 days before beat stress (2—3 days after surgery) until 20 days after termination of heat stress.

Heat stress and experimental design

The boars were exposed to heat stress in a temperature controlled room (climatic room) which was about 2.8 m×2.8 m, i.e. somewhat larger than the original pens. Duration of exposure was 100 h and the boars were then returned to their original pens. Four boars (experimental group) were exposed to 35°C and 40 % relative air humidity and 4 boars (control group) were kept in the climatic room at 20°C and 60—70 % relative air humidity. The conditions for the control group resembled as far as possible the normal barn environment. Semen collections were not performed in the climatic room.

All the boars were slaughtered when ejaculate sperm morphology had returned to normal and the genital organs were carefully examined post mortem. The results of semen examinations and post-mortem examinations will be published elsewhere (*Larsson & Einarsson*, to the published).

Statistical analysis

In the analysis of data the observational time was divided into 5 periods. Period 1 (days —5 to —1) was the period before heat stress, period 2 (days 1—5) was the period of heat stress and periods 3, 4 and 5 were the post-exposure periods.

The effect of treatment, period, day within period and time of sampling were estimated simultaneously by the method of least-squares analysis of data with unequal subclass numbers, using the Statistical Analysis System (*Helwig & Council* 1979). The following model was assumed to describe the data:

$$y_{ijklmn} = \mu + t_i + a_{ij} + p_k + tp_{ik} + d_{kl} + s_m + e_{ijklmn}$$

where

y_{ijklmn} = the $ijklmn$th observation
μ = general mean
t_i = effect of the ith treatment ($i = 1,2$)
a_{ij} = effect of the jth animal within the ith treatment
p_k = effect of the kth period of time ($k = 1,2,\ldots,5$)
tp_{ik} = effect of the interaction between the ith treatment and the kth period..
d_{kl} = effect of the lth day within the kth period
s_m = effect of the mth sample during the same day ($m = 1,2,3$)
e_{ijklmn} = residual random term with variance σ_e^2

The effect of animal was regarded as random and all other effects as fixed.

RESULTS

Fig. 1 shows the daily least-squares mean levels of testosterone for the two groups of boars. During period 1 the testosterone levels were similar for both groups of boars. During the stay in the climatic room (period 2) the testosterone levels differed significantly between the 2 groups of boars (Table 1). The control boars had significantly higher testosterone levels during this period than during any other period. The experimental boars had

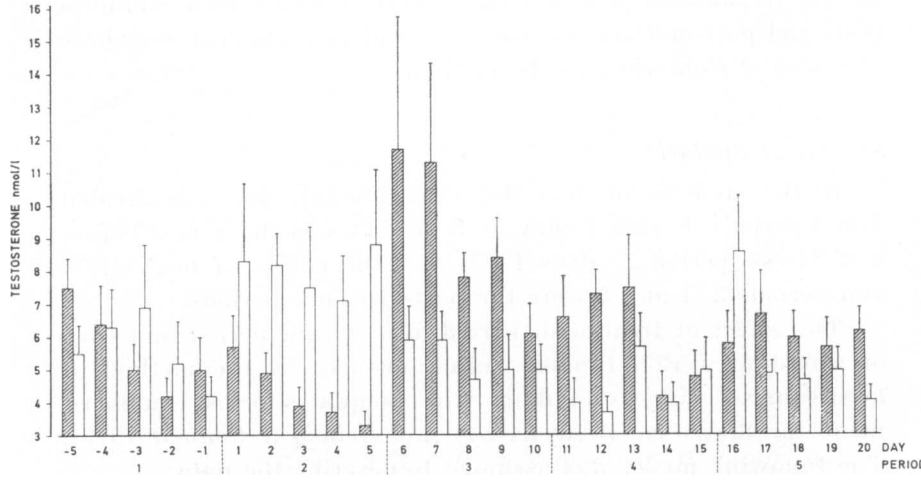

Figure 1. Daily least squares mean levels of testosterone for heat stressed (hatched bars) and control (open bars) boars. Vertical range bars indicate s.e.m. Period 2 was the time spent in the climatic room.

Table 1. Peripheral plasma levels (least-squares means ± standard errors) of testosterone and cortisol in boars before (period 1), during (period 2) and after (periods 3, 4, 5) treatment in climatic room.

Period (each 5 days)	Testosterone, nmol/l			Cortisol, nmol/l		
	Temperature in climatic room		Level of significance for the difference	Temperature in climatic room		Level of significance for the difference
	20°C	35°C		20°C	35°C	
1	$5.5^{a}\pm0.5$	$5.7^{ac}\pm0.5$	n.s.	$29.1^{ab}\pm3.2$	$30.9^{a}\pm3.2$	n.s.
2	$8.2^{b}\pm0.6$	$4.4^{c}\pm0.5$	***	$35.6^{b}\pm3.5$	$46.1^{b}\pm3.3$	*
3	$5.4^{a}\pm0.5$	$9.1^{b}\pm0.5$	***	$30.6^{ab}\pm3.2$	$27.2^{a}\pm3.1$	n.s.
4	$4.4^{a}\pm0.5$	$6.3^{a}\pm0.5$	**	$35.5^{b}\pm3.1$	$28.8^{a}\pm3.3$	n.s.
5	$5.6^{a}\pm0.5$	$6.0^{a}\pm0.5$	n.s.	$25.1^{a}\pm3.1$	$25.2^{a}\pm3.2$	n.s.

Levels of significance: n.s. (not significant): $P > 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Period means (within temperature treatment) with the same letter are not significantly different ($P > 0.05$).

slightly increased testosterone levels during the first day of heat stress and thereafter continuously decreased levels.

After removal from the climatic room the testosterone levels of the control boars returned to pretreatment levels (Table 1, Fig. 1). The heat-stressed boars had dramatically increased testosterone levels during the first 5 days after removal from the climatic room. According to Fig. 1 and Table 1 their testosterone levels exceeded pre-exposure levels for at least 5 days and remained thereafter at pre-exposure levels.

Fig. 2 shows the plasma levels of cortisol for the two groups of boars. The differences between the groups were restricted to the period spent in the climatic room (period 2). During this period the experimental boars had significantly higher plasma cortisol levels. Furthermore, the cortisol levels during exposure to heat stress were higher than during any other period in this group of boars (Table 1). From Fig. 2 it is evident that the most pronounced cortisol increase occurred during the first day of exposure (day 1).

Table 2 summarizes the levels of significance for the effects studied. For both hormones measured, the effects of animal and period on the peripheral plasma levels were significant. For both hormones the level in one of the daily samples differed significantly from the others. The lowest cortisol levels were found in

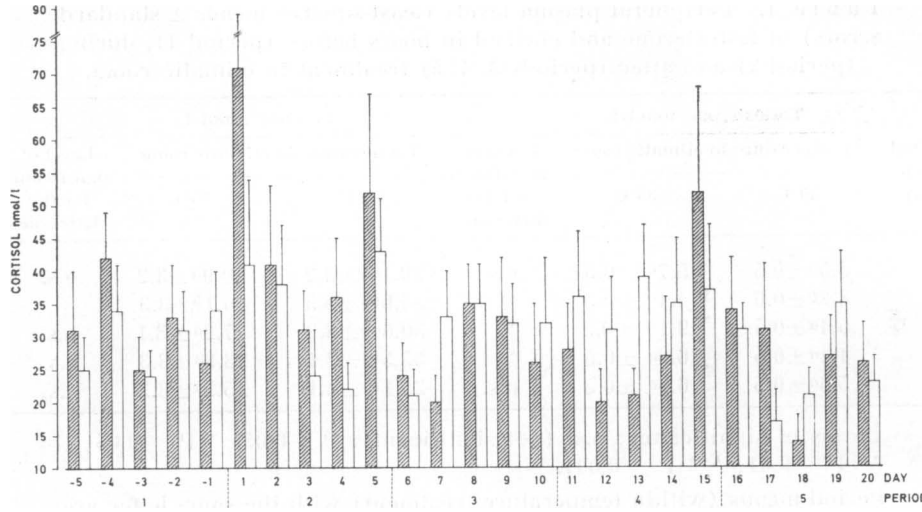

Figure 2. Daily least squares mean levels of cortisol for heat stressed boars (hatched bars) and control boars (open bars). Vertical range bars indicate s.e.m., period 2 was the time spent in the climatic room.

samples taken at 3 pm, while testosterone levels were highest in samples taken at noon. The differences due to time of sampling were much more expressed for cortisol than for testosterone.

Table 2. Levels of significance for the effects studied.

	Level of significance for					
Hormone	Treatment	Animal	Period	Treatment × period	Day	Sample
Testosterone	n.s.	***	*	***	n.s.	*
Cortisol	n.s.	***	*	n.s.	**	***

Levels of significance: n.s. (not significant): $P > 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

DISCUSSION

The present study was undertaken to investigate the temporal changes in plasma levels of cortisol and testosterone of boars during and after heat stress. Transfer of boars between herds is customary in many countries and most often it takes place when the boar is sexually mature and ready to be used for breeding. Thus stress, by change of environment, is likely to influence

many boars shortly before they start breeding. Heat stress is a type of environmental change that can be standardized and repeated with several animals and therefore this was used as an experimental model. Furthermore under Swedish climatic conditions semen production is reduced during the warmest period of the year (*Einarsson* 1968).

From the literature it is clear that the boar reacts to ACTH-injections with elevated plasma levels of cortisol and testosterone (*Liptrap & Raeside* 1975, *Hahmeier et al.* 1980, *Juniewicz & Johnson* 1981). The increased testosterone secretion does not seem to be mediated via LH (*Juniewicz & Johnson* 1981). Also after a short-time stress plasma levels of cortisol and testosterone are increased (*Liptrap & Raeside* 1978).

The present results indicate clear differences in hormonal patterns between the 2 treatment groups. These differences were most likely related to the heat stress, although the individual animal had a significant influence on hormonal levels. The influence of the individual was, however, partly overcome by the comparison of pre-treatment, treatment and post-treatment hormonal levels within the groups of boars.

The control boars, that where not exposed to high temperature while kept in the climatic room, remained unaffected except for an increase in testosterone levels during the time in the climatic room. This might be an effect of the change of housing, similar changes were reported in one boar after environmental change (*Claus & Alsing* 1975).

The experimental boars, exposed to high temperature for 100 h, showed decreased testosterone levels during heat stress in accordance with previous studies (*Wetteman & Desjardin* 1979, *Einarsson & Larsson* 1980). Simultaneously their plasma cortisol levels were significantly increased. This indicates that also in the boar a prolonged period of increased cortisol secretion will lead to decreased testosterone levels in the peripheral circulation. In the beginning of heat stress a slight increase in testosterone levels was found, indicating an initial similarity to a short-time stress. In men (*Doerr & Pirke* 1976), bulls (*Welsh et al.* 1979) and rats (*Bambino & Hsueh* 1981) corticoids have been found to suppress testosterone secretion. In bulls (*Welsh et al.* 1979) prolonged elevations in cortisol levels coincided with basal levels of LH and testosterone. The present study cannot be used to evaluate the temporal relationships between cortisol and

testosterone levels. However, it seems most likely that the boar reacts to stress similarly to other species with the exception of short time stress situations.

The dramatic increase in testosterone levels found after termination of heat stress is similar to the results of a previous study, under similar conditions but with other boars, in our laboratory (*Einarsson & Larsson* 1980). This occurred as the cortisol levels decreased to pre-exposure levels. The testosterone increase occurred only in boars where testosterone levels were reduced during heat stress. No similar tendency was found among the control boars or in boars exposed to heat stress for only 24 h with the exception of 1 animal (*Einarsson & Larsson* 1980). Thus the new change of environment as such does not seem to be the cause and furthermore all boars were returned to their original pens which most likely would still be familiar to them. Thus it seems likely that the testosterone rise observed represents a compensation for the period of reduced secretion.

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SAMMANFATTNING

Endokrina effekter av värmestress hos galtar

Ändamålet med föreliggande undersökning var att beskriva förändringar i perifera plasmanivåer av testosteron och cortisol hos galtar under och efter värmestress. Totalt ingick 8 galtar i försöken. Fyra av dem exponerades för 35°C under 100 timmar i en klimatkammare, medan 4 galtar användes som kontroller med 20°C temperatur under 100 timmar i klimatkammaren.

Blodprover uttogs via permanent inopererade venkatetrar tre gånger dagligen med början 5 dagar före värmestressen. Provtagningarna pågick sedan till dess att 20 dagar förflutit sedan värmestressen avslutats. Testosteron bestämdes med hjälp av radioimmunologisk teknik och cortisol med proteinbindningsteknik.

Nivåerna av cortisol och testosteron var lika i båda grupperna av galtar före vistelsen i klimatkammaren. Kontrollgaltarna hade signifikant högre testosteronnivåer under vistelsen i klimatkammaren än under någon annan del av observationstiden. Försöksgaltarna hade lätt stegrade testosteronnivåer under första dagen i klimatkammaren och därefter sjönk deras testosteronnivåer successivt. Kontrollgaltarnas testosteronnivåer återgick till de ursprungliga så snart de lämnat klimatkammaren. Hos de galtar som exponerats för 35°C, sågs under de första dagarna efter värmestressen kraftigt stegrade testosteronnivåer Skillnaderna i cortisolnivåer mellan grupperna av galtar inskränkte sig till tiden i klimatkammaren, där de galtar som exponerades för 35°C, hade signifikant höjda cortisolnivåer.

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Reprints may be requested from: Kjell Larsson, the Department of Obstetrics and Gynaecology, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden.