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Effects on Testosterone, LH and Cortisol Concentrations, and on Testicular Ultrasonographic Appearance of Induced Testicular Degeneration in Bulls

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Sidibé, M., L.A. Franco, G. Fredriksson, A. Madej and L. Malmgren: Effects on testosterone, LH and cortisol concentratrions, and on testicular ultrasonographic appearance of induced testicular degeneration in bulls. Acta vet. scand. 1992, 33, 191-196. – It is well known that heat stress has a detrimental effect on testicular functions. In addition to the alteration of semen quality and testicular damage, reproductive hormone secretion can be altered. The objective of this study was to decribe changes in plasma concentrations of testosterone, LH and cortisol, as well as in testicular ultrasonographic appearance after induced testicular degeneration. Four Swedish Red and White bulls, aged 3 years, were used. They were fed according to Swedish standards. The scrotum was covered with an insulation device during 96 h. Semen was collected weekly 3 times before and up to 4 months after insulation. Testicular ultrasonography and clinical genital examination were performed with the same intervals. Heparinized blood samples were taken from the jugular vein at 2 h interval during 24 h every 2 weeks during the study. Blood samples were tested for the content of testosterone, LH and cortisol. Data were analysed, using oneway analysis of variance of seminal data, clinical examination data as well as 24 h hormonal output data as perentage of mean individual pretreatment values. The use of a 5 MHz B-mode ultrasound unit did not contribute with an objective estimation of the degree of testicular degeneration. In 3 of the bulls testosterone levels had a tendency to decrease and LH to increase during the time of severe degeneration, whereas an opposite trend was seen during the regenerative phase, changes becoming significant 15 weeks after scrotal insulation. Variation between animals was big. Cortisol levels had a decreasing trend, changes being significant only in individual bulls at 10 and 15 weeks after scrotal insulation. One bull had to be treated separately as testosterone and LH patterns were deviating. It was concluded that severe testicular degeneration induces related changes in the levels of testosterone and LH.

heat stress; scrotal insulation.

Introduction

High temperature is an important environmental factor of testicular disorders. This was observed already in 1934 by *Lagerlöf.* More than 10 years ago *Ross & Entwistle* (1978) described that scrotal insulation in bulls altered semen quality as well as its correlation to the histological appearance of the seminiferous epithelium. These results are in agreement with the results of *Malmgren* (1988) in the boar. *Malmgren* also observed temporary changes of the plasma concentration of testosterone, oestradiol-17ß and oestrone sulphate. *Prabhakar et al.* (1989)

showed that the plasma concentration of testosterone decreases after scrotal insulation in bulls but could not observe any alteration of the LH levels. Prolonged stress in man was found to have a suppressive effect on plasma testosterone concentration *(Aakvaag et al.* 1978). Heat stress also has an effect on cortisol levels. *Aakvaag et al.* (1978) and *Larsson et al.*(1983) showed that prolonged stress lead to increased cortisol levels and at the same time to decreased testosterone levels. In boars,treated with ACTH for 5 days,reduced testosterone concentrations were observed *(Liptrap & Raeside* 1975). The latter studies indicate, that in addition to the alterations of the semen quality, the reproductive hormones can be used to evaluate the testicular disorders. In order to further clarify this, the objectives of the study were to

- experimentally induce thermal testicular degeneration by means of scrotal insulation,
- describe the temporal changes in the peripheral plasma concentration of testosterone, cortisol and LH,
- describe the ultrasonographic appearence of the testicles,
- correlate this to the semen quality and clinical genital findings.

Materials and methods

Animals

4 Swedish Red and White bulls aged 3 years were used in the study. The animals were kept in the barn of the Department of Obstetrics and Gynaecology, and fed a standard ration of concentrate and straw-hay.

Scrotal insulation

The scrotum was covered with an insulation device, a double plastic bag with a cotton cloth inside. The duration of the scrotal insulation (SI) was 96 h (4 days). During SI, the scrotal skin temperature was checked once daily with an electric universal thermometer (Swematemp 180, Farsta, Sweden).

Semen collection and evaluation

Semen was collected in an artificial vagina weekly before SI and until 10 weeks after SI as well as at 12 and 15 weeks. Immediately following collection, the semen was evaluated according to standard procedures for volume, motility, massactivity, concentration and morphology.

Clinical genital examination

The clinical examination was made before and after SI once weekly. This examination of the testes comprised size, firmness and consistency. A score was given according to the scoring system of *Galloway* (1969). For the epididymis, the contents and consistency were checked. The length and the breadth of each testis were measured using a ruler. Scrotal circumference was measured with a scrotal tape.

Ultrasonography

Before SI and once weekly after SI, the testicular appearence was measured with a 5 MHz B-mode ultrasound unit (Aloka Co. Ltd, Japan). Ultrasound transmission gel was applied on the scrotum. Each testis was scanned sagitally and transversally in order to follow the testicular echogenicity changes.

Blood sampling

The blood samples were taken in the jugular vein using heparinized vacutainer tubes. The sampling was done before SI and 6 times after SI at 2-weeks interval as well as at 15 weeks after SI, the first sampling after SI being the same day when the insulation bags were taken off. Samples were taken every 2 h

during 24 h, ie. 13 samples each time. The blood was centrifuged immediately and the plasma harvested and stored at –20°C.

Hormone assay

Plasma concentrations of testosterone were determined by solid-phase ^{125}J radioimmunoassay (Coat-A-Count Diagnostic Products Cooperation, USA). Cortisol was measured by a competitive immunoassay technique based on enhanced luminescence (The Amerlite Cortisol Assay, Amersham, UK). Both testosterone and cortisol assay were previously validated for bovine plasma. Plasma LH concentrations were measured by homologous radioimmunoassay as described by *Madej et al.* (1989).

Statistical analysis

The handling and analysis of the data were performed using the Statgraphic programme (STSC,Inc.,Rocheville,MO,USA). The 24 h variations of testosterone, LH and cortisol were estimated as the area under the curve using an electronic integrator (Digiplan, Kontron Messgeräte, Germany). Data from semen and clinical evaluation were transformed into mathematical terms using a scoring system. The hormonal and semen changes were evaluated by a one-way analysis of variance and confidence interval test.

Results

The mean testicular skin temperature was 31.6°C before SI and the mean temperature inside the insulation device during SI was 34.8, 35.5, 35.8, 36.0 and 35.8°C within 2, 24, 48, 72 and 96 h after insulation. Thus, a temperature increase of 3.2 - 4.4°C was maintained during 96h.

Fig. 1 shows the clinical genital and seminal changes in 3 of the 4 bulls, the scores being significantly lower from 2 weeks after SI up to the end of the study. Large individual variation was noted. The fourth bull followed the same pattern but was deleted from the figure.

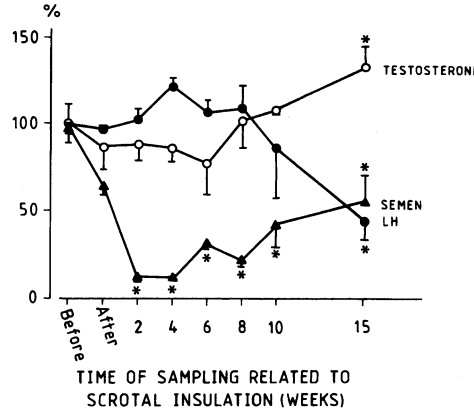

Figure 1. Changes in plasma levels of testosterone and LH (estimated as the area under the curve), and in clinical genital score ("semen") after scrotal insulation. This is expressed as percent changes compared to the mean individual pretreatment values. *indicates statistical significant difference ($p \leq 0.05$).

The ultrasonography measurements of the testicular appearance before and after SI did not reveal any objective changes. The echogenicity did not follow neither the clinical nor the seminal changes in a logical way, this irrespective of if a correlation was done directly in time or with a displacement. The results were dependent on the operator and on the way of application of the probe.

The testosterone and LH values of 3 of the bulls before and after SI are presented in Fig. 1 as the relative area under the curve. Immediately after SI and during 6 weeks, the 3 bulls showed a tendency to a decrease in testosterone levels and an increase in LH levels. After 8 weeks, testosterone levels increased and LH decreased. The tendency for testosterone became significant by 15

weeks compared to its lowest values at 6 weeks and for LH compared to the levels between 2 and 8 weeks after SI. In general, LH and testosterone peaks were correlated but at some occasions the peaks of testosterone appeared when no peaks of LH were seen and vice versa. The fourth bull had irregular peak patterns of LH and testosterone and could not be evaluated together with the other bulls.

In the 4 bulls together no significant changes of the cortisol levels were observed. However, this hormone showed a general tendency to decrease and the changes became significant in the 2 last sampling occasions analysing individual bulls.

Discussion

The temperature obtained inside the insulation bag (35.5°C) is close to that reported by *Ross & Entwisthle* (1979) in the bull and by *Malmgren* (1988) in the boar.

The changes of the semen quality as well as the clinical genital changes were in agreement with previous reports *(Lagerlöf* 1934, *Ross & Entwisthle* 1978, *Randall* 1981, *Blanchard et al.,* 1991 a,b).

The use of a 5 MHz B-mode ultrasound unit in order to follow changes in the testicle during testicular degeneration did not reveal any objective changes of the echogenicity before and after SI. Also *Eilts & Pechman* (1988) tried to relate ultrasonographic findings to seminal characteristics and testicular tissue damage during andrological examination of bulls, but no significant correlations were obtained. On the other hand ultrasound may enhance the diagnosis of focal or multifocal diseases such as fibrous scarring, neoplasia or abscesses.

The testosterone levels in 3 of the bulls showed a decline immediately after SI and then started to increase 2 weeks later. The decrease of testosterone levels was also observed by *Prabhakar et al.* (1989) in the bull, by *Malmgren* (1988) in the boar and by Blanchard et al. (1991 a,b) in the ram. In addition *Rhynes & Ewing* (1973) observed an increase after 3 weeks while *Prabhakar et al.* (1989) reported 1 week. In the boar, the testosterone levels increased after 1 to 4 days *(Malmgren* 1988). This absolute increase soon after SI was not detected in this study perhaps due to infrequent bloodsampling.

The LH secretion is following that of testosterone but in the opposite direction. In man, *Kretser et al.* (1989) observed a decrease of testosterone levels and at the same time LH was increasing during a severe spermatogenic damage. This finding is similar to the present results. By looking at the curves of LH and testosterone for each bull separately, the testosterone peaks appear when no peaks of LH were seen and vice versa. The feedback mechanism of testosterone regulation seemed disturbed and seemed not to be the only component involved in the testicular regulation. This observation is in agreement with *Amann* (1961), *Byers & Glover* (1984) and *Bellvé & Zheng* (1989). They claim that paracrine signals are involved in a "testicular triangle" of communication among *Leydig* cells, *Sertoli* cells and germ cells. This testicular control of *Leydig* cell steroidogenesis can be one explanation of the disturbances observed in the hormonal profiles of these bulls. It might also explain the significant compensatory increase in testosterone at 15 weeks when the semen picture was not yet completely normalized.

As regards the cortisol, a general decreasing tendency was observed for this hormone. This phenomenon might be the effect of the season as the blood was collected from summer to autumn. This is in agreement with

Lamothe-Zavaleta (1990), who found that the cortisol levels are high in the rainy season (the season of climatic stress) and low in the dry season. Another explanation is that the animals got more used to the handling and to the blood sampling as such.

The present findings suggest that there is a possibility of describing at least severe testicular degeneration by monitoring reproductive hormones such as testosterone and LH. However, for the bull it is necessary to improve the technique of experimentation and to go deeper into the neuro-paracrine system of the testes.

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References

Aakvaag A, Bental O, Quigstad K, Walstad P, Ronningen H, Fonnum F: Testosterone and testosterone binding globuline (TeBG) in young men during prolonged stress. Int. J. Andr. 1978, *1,* 22-31.

Amann RP: How a bull works. Proc. 11th. Technical Conference on Artificial Insemination and Reproduction. 1986, pp. 6-18.

Bellvé AR, Zheng W: Growth factors as autocrine and paracrine mediators of male gonadal function. J. Reprod. Fert. 1989, *85,* 771-793.

Blanchard TL, Varner DD, Bretzlaff KN, Elmore RG: The causes and pathologic changes of testicular degeneration in large animals. Vet. Med. 1991a, *86,* 531-536.

Blanchard TL, Varner DD, Bretzlaff KN, Elmore RG: Testicular degeneration in large animals: Identification and treatment. Vet. Med. 1991b, *86,* 537-542.

Byers SW, Glover TD: Effect of scrotal insulation on the pituitarytesticular axis of the ram. J. Reprod. Fert. 1984, *71,* 23-31.

Eilts BE, Pechman RD: B-mode ultrasound observations of bull testes during breeding soundness examinations. Theriogenology 1988, *6,* 1169-1175.

Galloway D: Compendium, Swedish International Programme on Animal Reproduction, Swedish University of Agricultural Sciences. Uppsala, Sweden. 1969.

Kretser de DM, McLachlan RI, Robertson DM, Burger HG: Serum inhibin levels in normal men and men with testicular disorders. J. Endocrin. 1989, *120,* 17-523.

Lagerlöf N: Morphologische untersuchungen über veränderungen im spermabild und in den hoden bei bullen mit verminderter oder aufgehobener fertilität (Morphologic investigations of changes in sperm and testicle in bulls with reduced or ceased fertility). PhD thesis, Stockholm, Sweden 1934, 254pp.

Lamothe-Zavaleta C: Reproductive performance of zebu cattle in Mexico. MSc thesis. Swedish University of Agricultural Sciences. Uppsala, Sweden. 1990.

Larsson KS, Einarsson S, Lundström K, Hakkarainen J: Endocrine effects on heat stress in boars. Acta vet. scand. 1983, *24,* 305-314.

Liptrap RM, Raeside JI: Increase in plasma testosterone concentration after injection of adrenocorticotrophin into the boar. J. Endocrin. 1975, *66,* 123-131.

Madej A, Hallin P, Madej M, Seguin B, Edqvist L-E: Influence of bovine LH tracer quality on levels of LH in GnRH-treated cows. J. Immunoassay 1989, *10,* 277-300.

Malmgren L: Experimentally induced testicular alterations in boars. MSc Thesis. Swedish University of Agricultural Sciences. Uppsala, Sweden. 1988.

Prabhakar J, Chimbombi M, Malmgren L, Fredriksson G, Madej A: Effects on testosterone and LH concentrations of induced testicular degeneration in bulls. Acta vet. scand. 1990, *31,* 505-507.

Randall S: How to examine bulls for breeding soundness. Proceedings of the annual meeting. Society for Theriogenology 1981, pp. 1-15.

Rhynes W E, Ewing L L: Testicular endocrine function in Hereford bulls exposed to high ambient temperature. Endocrinology 1973, *92,* 509-515.

Ross AD, Entwistle KV: The effect of scrotal insulation on spermatozoal morphology and the rates of spermatogenesis and epididymal passage of spermatozoa in the bull. Theriogenology 1979, *11,* 111-121.

Sammanfattning

Påverkan på plasmakoncentrationerna av testosteron, LH och kortisol samt på testikelvävnadens ultraljudskarakteristika, efter inducerad testikeldegeneration hos tjur.

Det är välkänt att värmestress negativt påverkar testikelfunktionen. Förutom den direkta testikelskadan och påverkan på spermabilden kan vissa reproduktionshormoner påverkas. Avsikten med denna studie var att beskriva förändringen i plasmakoncentrationerna av testosteron, LH och kortisol liksom förändringar av testikelvävnadens utseende på ultraljud efter en inducerad testikeldegeneration.

Fyra treåriga svenska röda och vita tjurar användes. De uppstallades vid institutionen och utfodrades enligt svensk standard. Skrotum täcktes av en isoleringspåse under 96 h. Spermaprov togs varje vecka 3 gånger före och upp till 4 månader efter skrotumisoleringen. Ultraljuds- och klinisk andrologisk undersökning utfördes med samma intervall. Hepariniserat blod togs från jugularvenen varannan timme under 24 timmer varannan vecka. Blodproven analyserades med avseende på testosteron, LH och kortisol. Data från spermaprover, kliniska undersökningar och hormonanalyser överfördes till procentuella förändringar i förhållande till medelutgångsvärdena och analyserades statistiskt med hjälp av variansanalys.

Ultraljud, i form av en 5 MHz B-mode-apparat, kunde inte användas som ett objektivt bedömningssätt av testikeldegeneration.

Hos 3 av tjurarna hade testosteronnivåerna en tendens att minska och LH-nivåerna att öka under den tid då testikeldegeneration var som mest uttalad. Under regenerationsfasen sågs en motsatt tendens, vilken blev signifikant 15 veckor efter skrotumisoleringen. Variationen mellan tjurarna var stor. Kortisol hade en nedåtgående trend och förändringarna blev signifikanta endast för de enskilda tjurarna vid 10 och 15 veckor. En tjur måste anlyseras separat pga avvikande testosteron- och LH-mönster. Slutsatsen kunde dras att grav testikeldegeneration orsakar förändringar i testosteron- och LH-nivåerna.

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