Acta vet. scand. 1976, *17*, 475—487.

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5α-ANDROSTENONE AND TESTOSTERONE IN PERIPHERAL PLASMA OF THE BOAR DURING AND FOLLOWING COPULATION*

By

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ANDRESEN, ØYSTEIN: *5α-Androstenone and testosterone in peripheral plasma of the boar during and following copulation.* Acta vet. scand. 1976, *17*, 475—487. — Copulation was generally followed by increases in peripheral plasma 5α-androstenone and testosterone levels lasting for periods of about 60 to 100 min. The effect of copulation on the plasma levels of these steroids did, however, vary between boars. In seven out of eight boars the maximum levels of 5α-androstenone in the period 60 to 100 min. after copulation were from 114 to 218 % (mean 150 %) of the levels in samples collected before copulation. The corresponding figures for testosterone were from 104 to 283 % (mean 190 %). One boar showed decreasing plasma steroid levels after copulation.

The coefficient of correlation between the peripheral plasma levels of 5α-androstenone and testosterone was found to be + 0.61 (n = 203).

5α-androstenone; testosterone; copulation; sexual stimulation; boar.

Odorous steroids represent an important group among steroids secreted by the boar testes. Chemically they are C_{19} steroids with a double bond between C_{16} and C_{17}, and their biosynthesis

* This study was supported by the Norwegian Agricultural Research Council.

from pregnenolone* and progesterone is entirely different from the biosynthesis of androgens and oestrogens (for review see *Gower* 1972).

One of these steroids, 5α-androstenone has a prominent urine-perspiration-like odour. Interest in this steroid has been evoked for two reasons: First, due to its monopolar, lipophilic nature, it accumulates in the adipose tissue of the boar and makes up a main component of the boar taint or sex odour (*Patterson* 1968). This unpleasant odour can easily be detected in boars producing significant amounts of the steroid when fat taken from these animals is heated. The presence of the steroid in the carcass therefore represents an obstacle to the utilization of boar meat. Second, 5α-androstenone is excreted in the saliva of boars and may act as a pheromone or sex attractant affecting the reproductive behaviour of oestrous sows (*Melrose et al.* 1971, *Reed et al.* 1974).

The peripheral plasma levels of 5α-androstenone and testosterone increase during growth and sexual maturation in most boars, and previous studies have also indicated that sexual stimulation might affect the plasma levels of these steroids (*Andresen* 1976). The object of the present study was to obtain more detailed information on the short term effect of copulation on the peripheral plasma levels of 5α-androstenone and testosterone in the boar.

MATERIAL AND METHODS

Series 1

Eight Norwegian Landrace boars, 6 to 9½ months old, were studied. Six of the boars had previously been used for mating from 15 days to one day before they were used in this study (Fig. 1). The animals were penned individually in a room also housing a number of gilts and sows. Oestrous sows were released into the pens of five of the boars, whilst the remaining three

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* Abbreviations and trivial names used:
5α-androstenone: 5α-androst-16-en-3-one;
testosterone: 17β-hydroxy-4-androsten-3-one;
dihydrotestosterone: 17α-hydroxy-5β-androstan-3-one;
pregnenolone: 3β-hydroxy-5-pregnen-20-one;
progesterone: 4-pregnene-3,20-dione;
HCG: human chorionic gonadotropin;
LH: luteinizing hormone.

boars served as control animals and were not allowed physical contact with female animals. The sows were removed from the pens as soon as copulation, which lasted for about 5 min., had taken place. One of the boars (no. 4) was studied twice with an interval of 28 days during which the boar was used for five services.

All experiments were started at 10 a.m. With the exception of one case (boar no. 4^{I}, Fig. 1), blood samples were collected 5—20 min. before the boar was presented to the oestrous sow, once during the copulation and then 30, 60, 100, 160 and 220 min. afterwards. Additional samples were collected 24 and 48 hrs. after copulation. Samples were collected with the same time intervals in the control animals.

Series 2

The results from series 1 indicated that the response in plasma steroid levels to copulation was related to the extent to which the animals had been sexually stimulated during the days prior to study. In order to test this tentative conclusion, the effect of copulation on plasma steroid levels was studied in three 8—9 months old boars on three consecutive days. One of the boars, no. 9, had never previously been used for mating, while the others (nos. 10 and 11) were sexually experienced animals. In order to avoid the effect of the presence of other sexually mature pigs, the boars were placed one at a time in a pen in a room where, apart from the boar concerned, only piglets were present.

Samples of peripheral blood were collected daily at about 9 a.m. starting on the first day after the boars had been placed in the pens (day 1, Fig. 2). On the third day of the experiments seven additional control samples were collected at set intervals during the following 5 hrs. Later on, oestrous sows were let into the pens of the boars on three consecutive days. Copulation took place whereafter the sows were removed. Samples of peripheral blood were collected once during and 30, 60, 90, 120, 180, 240 and 300 min. after copulation.

The boars both in series 1 and 2 were included in a selection experiment (*Standal* 1967). Boars nos. 1, 2 and 4 belonged to a line (highpoint) selected for low back fat thickness and a high rate of gain, nos. 3, 5, 7 and 9 had been selected in the opposite direction (low point) while nos. 6, 8, 10 and 11 were boars from a line which had been maintained without deliberate selection

(control line). The levels of 5α-androstenone and testosterone in boars from these different lines have been studied previously (*Andresen & Bakke* 1975, *Andresen* 1976).

All samples were collected from ear veins into heparinized vacutainers. The damage to the vein was minimized by using 25 gauge needles, and development of haematomas was avoided by applying positive pressure at the site of puncture for 1—2 min. after sampling. Plasma was prepared by centrifugation and stored at —20°C until analysed.

With the exception of the samples collected during copulation, the boars were restrained by means of a rope around the snout during sampling.

Plasma levels of 5α-androstenone were estimated by radioimmunoassay (*Andresen* 1974). The crossreactivity of the antibodies with testosterone is less than 0.45 %. The efficiency of extraction of 5α-androstenone from plasma is 82.8 ± 4.4 % (s), n = 171, and this mean value was used in the correction for experimental losses.

Testosterone was estimated by the radioimmunoassay described by *Sanwal et al.* (1974) as modified by *Sundby et al.* (1975). The antiserum crossreacted with dihydrotestosterone to a degree of 40 %, but with 5α-androstenone the crossreactivity is less than 0.45 %. A mean efficiency of extraction of 90 % (s = 3.6 %, n = 120) was used as correction factor to calculate the concentrations of testosterone.

For the analyses of 5α-androstenone, the standard deviation of duplicate estimations was 1.89 ng/ml, corresponding to a coefficient of variation of 9.6 % of the mean value. The corresponding figures for the analyses of testosterone were 0.47 ng/ml and 6.2 % respectively.

RESULTS

Series 1

The variation in plasma steroid levels in the three control animals during a period of 220 min. was in general relatively slight (Fig. 1, boars nos. 1, 2 and 3), but analysis of variance of measurements within animals revealed significant variation in in 5α-androstenone concentration in boar no. 2 ($P < 0.01$) and in testosterone concentration in all three boars ($P < 0.05$ to $P < 0.001$). The coefficients of variation for the 5α-androstenone and testosterone concentrations in the seven samples from each

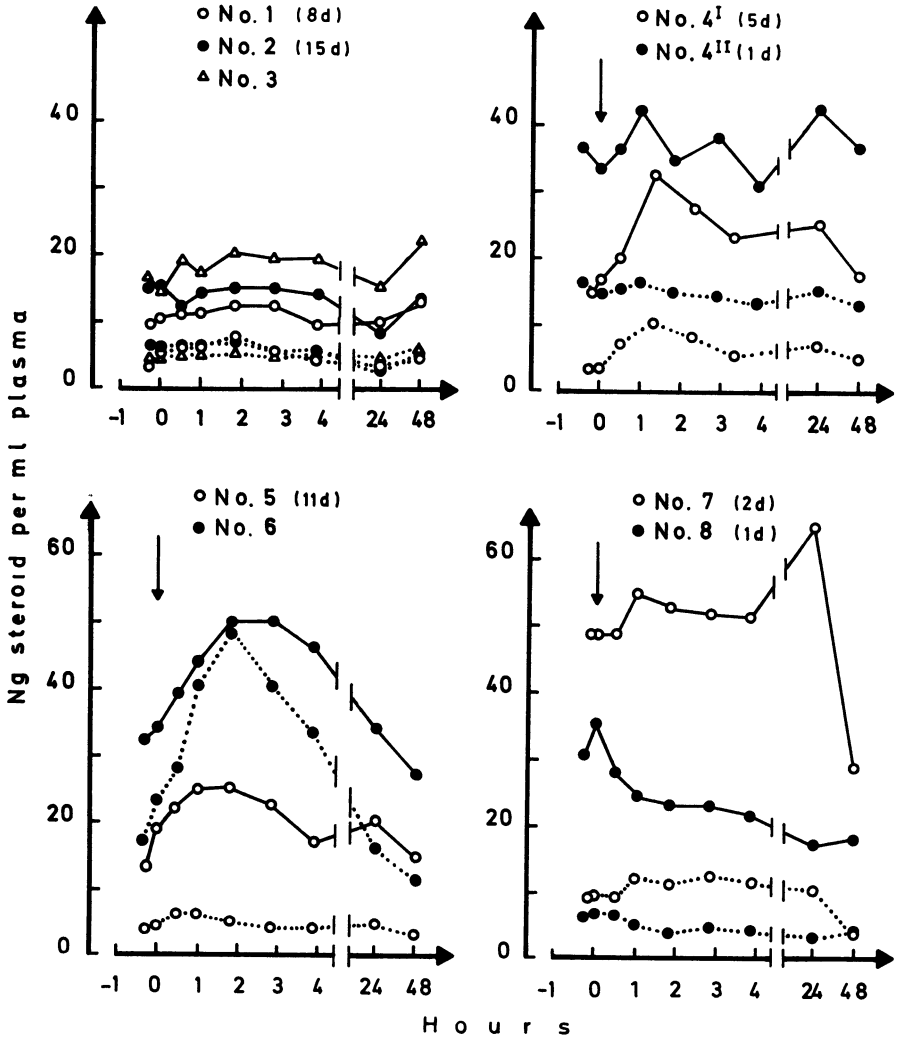

Figure 1. Concentrations (ng/ml) of 5α-androstenone (————) and testosterone (.....) in peripheral plasma of boars during and after copulation. The arrows denote copulation. Boars 1, 2 and 3 are control animals not allowed physical contact with female animals. The figures in brackets indicate days after last previous copulation. Boars no. 3 and no. 6 had not previously been used for mating.

of the three boars were found to be 11.1, 12.2 and 7.6 % and 26.0, 11.3 and 8.7 % respectively.

The plasma levels of steroids in samples collected before the boars were presented to oestrous sows varied markedly between

individual animals; 5α-androstenone ranging from 13.8 ng/ml to 48.5 ng/ml, and testosterone ranging from 4.0 ng/ml to 17.4 ng/ml.

In three out of the six cases, copulation markedly increased the levels of 5α-androstenone and testosterone (Fig. 1, boars nos. 4I, 5 and 6). The increase lasted for 60 to 100 min., whereafter decreasing levels were observed during the hours that followed. The maxima of 5α-androstenone were 154, 183 and 218 % of the levels found before copulation. The corresponding figures for testosterone were 155, 279 and 283 %, respectively. None of these boars had been used for mating the preceding few days.

In two cases the increase was less obvious (Fig. 1, boars nos. 4II and 7), and the levels of 5α-androstenone 60 min. after copulation were only 114 and 115 % and the testosterone levels 104 and 125 % of the levels in samples collected prior to copulation. The initial levels of steroids in these two boars were, however, rather high.

In one boar (Fig. 1, boar no. 8) copulation was followed by decreasing plasma levels of 5α-androstenone and testosterone.

In the boar studied twice (Fig. 1, boar no. 4) the general levels of the two steroids during the second experiment were substantially higher than during the first experiment, and the increase was less pronounced following the second copulation than the first.

No consistent relationship was observed between the steroid levels 24 hrs. after copulation and the levels recorded during the first hours after mating, but from 24 to 48 hrs. following copulation decreasing levels were observed in most animals.

Series 2

After moving the boars to the experimental location decreasing levels of plasma steroids were observed (Fig. 2).

The coefficients of variation for the 5α-androstenone and testosterone concentrations in samples collected during the control period (day 3, Fig. 2) were found to be 15.0, 10.4 and 6.8 % for 5α-androstenone and 30.0, 13.8 and 8.0 % for testosterone in boars nos. 9, 10 and 11 respectively. Analysis of variance revealed significant variation in 5α-androstenone concentration in boars nos. 10 and 11 ($P < 0.05$) and in testosterone concentration in all three boars ($P < 0.01$ to $P < 0.001$).

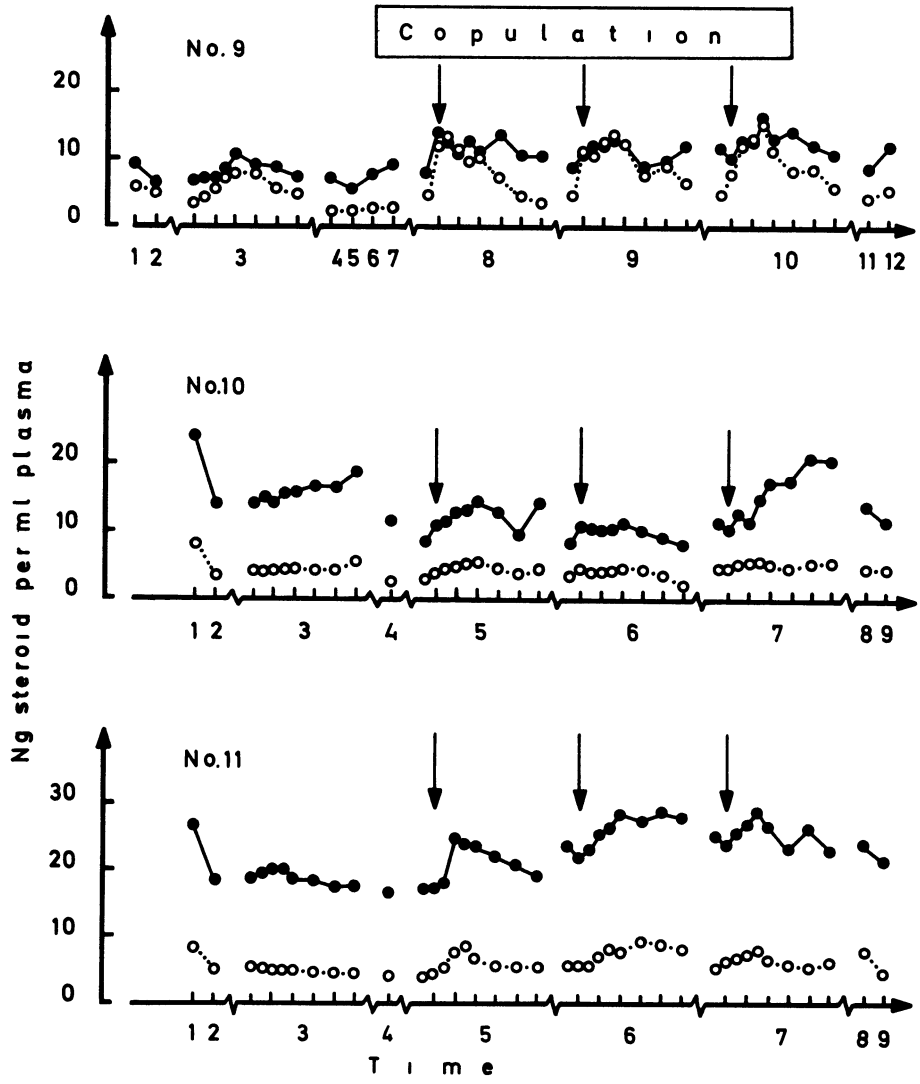

Figure 2. Concentrations (ng/ml) of 5α-androstenone (• ——— •) and testosterone (o ..... o) in peripheral plasma of three boars during and following copulation on three consecutive days. The figures on the time axis denote days, and the subdivision within days hours.

Copulation on three consecutive days did not seem to alter the effect on the steroid levels markedly (Fig. 2). The profiles did, however, differ somewhat between boars. In boar no. 9 the increase in steroid levels appeared very rapidly, and the concen-

tration of steroids in samples collected during copulation were in general found to be much higher than in the control samples collected about 30 min. previously. In this animal increasing peripheral plasma levels of steroids were also observed during the first 2 hrs. of the control period. This increase was not as marked as that seen in connection with copulation.

In boar no. 10, the response differed slightly from one copulation to the next. The third copulation was followed by a marked increase in 5α-androstenone levels while the testosterone remained at a fairly constant level.

In boar no. 11, all copulations were followed by increased peripheral plasma levels of both 5α-androstenone and testosterone.

In most instances the maximum concentrations of steroids were encountered within the same time interval as in series 1. The mean peripheral plasma levels of 5α-androstenone and testosterone in samples collected from the boars 90 min. after copulation were 138 % and 192 % of the levels in samples collected before copulation.

Neither in series 1 nor in series 2 did the results point to any great difference between lines of boars.

The levels of 5α-androstenone and testosterone in the total number of samples were found to be positively correlated, the coefficient of correlation being + 0.61 ($n = 203$).

DISCUSSION

The variation in 5α-androstenone levels in the control animals in series 1 and also during the control period in series 2 was in general found to be relatively slight, although the variation was statistically significant in three of the boars. Analysis of variance of measurements of testosterone within the same animals and periods revealed significant variation in all six boars, and especially in boars nos. 1 and 9 considerable fluctuations in plasma testosterone concentrations were observed.

Although a few exceptions were noticed, the results of the present studies indicate that in the boar, copulation is generally followed by increasing plasma levels of testicular steroids. This is in agreement with the findings reported by *Claus & Alsing* (1976) in a single animal. On the other hand, studies on minipigs (*Ellendorf et al.* 1975) revealed no significant changes in

plasma testosterone concentrations after copulation although a significant increase in plasma LH was observed.

The varying response seen in different animals in the present study seems to be slightly related to the general levels of steroids at the start of the mating tests, and marked increases were noticed in boars with rather different initial levels. The results from series 1 indicated that the varying response was related to the extent to which the animals had been sexually stimulated during the preceding days. However, this does not seem to be the case since repetitive copulations with 24 hrs. intervals did not change the response to any great extent.

As to the steroid levels following copulation in the different lines of boars, the number of animals is too small to draw final conclusions. It is, however, evident that the steroid levels vary to a considerable degree between animals within the different lines.

Restraint of the boars during sampling certainly represents a stress to the animals. This might have influenced the results, as corticosteroids are known to depress plasma LH activity in boars (*Liptrap & Raeside* 1968). An increased secretion of catecholamines might also have occurred, although it might be wrong to assume that all types of stress stimulate the sympathetic system. Nevertheless, it seems relevant to mention that in vitro, catecholamines have been found to increase adenyl cyclase activity in homogenates of testes (*Murad et al.* 1969). In vivo, however, injection of adrenalin has been found to decrease the peripheral plasma testosterone concentration in men (*Levin et al.* 1967). The most likely effect of restraint would therefore be a lowering effect on plasma steroid levels. Catecholamines will, however, also stimulate lipolyses, and this might affect the level of 5α-androstenone in peripheral plasma as adipose tissue in boars might contain microgram quantities of 5α-androstenone per g fat. Such a mechanism could explain the increasing levels of 5α-androstenone and the relatively stable peripheral plasma levels of testosterone following the third copulation in boar no. 10.

The effect of sexual stimulation on the peripheral plasma levels of testicular steroids has been studied in a number of other species e.g. man (*Fox et al.* 1972, *Stearns et al.* 1973, *Lee et al.* 1974), bull (*Katongole et al.* 1971, *Smith et al.* 1973), rabbit (*Saginor & Horton* 1968, *Haltmeyer & Eik-Nes* 1969, *Blake et al.* 1975, *Hilliard et al.* 1975), stallion (*Ganjam & Kenney* 1975) and

ram (*Purvis et al.* 1974, *Illius et al.* 1976). Although *Fox et al.* found that copulation was associated with a significant increase in plasma testosterone in one human male, this finding has not been confirmed in other studies in men, and *Lee et al.* concluded that "there is no evidence that coitus increases plasma testosterone". In the ram, the testosterone levels were not found to be affected by copulation (*Purvis et al.*). However, this observation was made at a time of the year when testicular activity was low. During the breeding season marked increases in plasma testosterone have been observed in some animals after copulation (*Illius et al.*). In the bull, *Katongole et al.* found that sexual stimulation caused LH release which was followed by increased levels of plasma testosterone if the testosterone level was low, but unaffected if the level was high at the time of the LH release. The increase in testosterone levels in rabbits after copulation is also most marked in animals with low initial levels (*Blake et al.*), and *Hilliard et al.* observed decreased levels in animals in which the initial levels were high.

The copulation-induced increase in the peripheral plasma levels of 5α-androstenone and testosterone in boars seems to be comparable to the first, rapid increase observed following intravenous HCG administration (*Andresen* 1975, *Carlstrøm et al.* 1975). As is evident from the 24 hrs. observations, copulation does not, however, seem to have any effect comparable to the prolonged effect of HCG, observed as increases in testicular steroid levels taking place 5 to 30 hrs. after intravenous injection.

Increases in the plasma levels of 5α-androstenone will rapidly affect the levels in adipose tissue. *Malmfors et al.* (1976) found that the levels of 5α-androstenone in fat doubled from about 1 μg per g to about 2 μg per g within 24 hrs. following intravenous HCG injection. It thus seems reasonable to presume that even the short term effect of a single copulation might significantly affect the level in adipose tissue of some animals, although the concentration of 5α-androstenone in adipose tissue has been found to be only slightly affected by the number of services which the boar has performed (*Andresen & Bakke* 1975).

ACKNOWLEDGEMENTS

I should like to express my sincere thanks to Miss Kari Bentzen for her skilful technical assistance, to Dr. N. Standal for providing the animals and to Dr. Anne Sundby for providing antibodies against testosterone.

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SAMMENDRAG

5α-Androstenon og testosteron i perifert plasma hos råne under og etter parring.

Etter parring så en som regel stigning i plasmakonsentrasjonen av 5α-androstenon og testosteron i et tidsrom av 60 til 100 minutter. Effekten av parring varierte imidlertid fra dyr til dyr. Hos 7 av 8 dyr fant en at de høyeste konsentrasjonene av 5α-androstenon i tidsrommet 60 til 100 minutter etter parring utgjorde fra 114 til 218 % (Middel 150 %) av konsentrasjonene i prøver tatt før parring. De tilsvarende verdier for testosteron var fra 104 til 283 % (Middel 190 %). Hos en råne fant en en nedgang i konsentrasjonene av steroider etter parring. Korrelasjonseffisienten mellom konsentrasjonene av 5α-androstenon og testosteron i perifert plasma var + 0.61 (n = 203).

(Received November 8, 1976).

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