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PLASMA TESTOSTERONE IN COWS RELATED TO DAY OF GESTATION AND SEX OF THE FOETUS, AND PLASMA TESTOSTERONE LEVELS AROUND PARTURITION

By

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SUNDBY, A. and Ø. JOAKIMSEN: *Plasma testosterone in cows related to day of gestation and sex of the foetus, and plasma testosterone levels around parturition.* Acta vet. scand. 1982, *23*, 438—445. — No significant differences in plasma testosterone level were observed between cows carrying a male foetus and cows carrying a female foetus at any ten-day interval from day 35 of gestation until parturition. Reported higher abortion rates for male than for female foetuses would thus appear not to be due to effects of foetal testosterone on the maternal endocrine balance. In spite of a great individual variation in plasma testosterone values at similar stages of gestation, certain trends are evident. From the 35th to the 80th day of gestation the average concentration was 90—100 pg/ml. Later it rose and reached 200 pg/ml on the 180th day, remaining at this level until after partus. During the first day after parturition plasma testosterone fell significantly and stabilized around 120 pg/ml.

Cow; gestation; parturition; plasma testosterone; foetal sex.

Low fertility in Norwegian Red Cattle was found to be followed by low percentage of bull calves (*Skjervold & James* 1979) indicating that embryonic death is more frequent among males than females. An explanation might be that the male foetus could induce its own abortion by negative feedback from foetal testosterone on maternal LH which would subsequently reduce stimulation of maternal progesterone production.

Through in vivo and in vitro studies of various species and castration in early foetal life, it appears that sexual differentiation to a large extent depends on foetal steroids (*Jost* 1947, *Attal* 1969, *Wilson & Siiteri* 1973, *Raeside* 1974, *Warren et al.* 1975). The foetal testes of sheep and rabbit produce testosterone from a few days prior to the time when sexual differentiation is possible (*Attal, Wilson & Siiteri*). Sexual differentiation in the male bovine foetus occurs from about the 43rd day of gestation and significantly higher plasma testosterone is reported in the male than in the female foetus (*Kim et al.* 1972, *Mongkonpunya et al.* 1975). At an unspecified day of gestation, *Kim et al.* measured significantly higher plasma testosterone in 9 pregnant than in 6 non-pregnant cows. Reports differ concerning the influence of foetal sex upon the maternal plasma testosterone level (*Kim et al., Mongkonpunya et al., Hoffmann et al.* 1976).

The present investigation was undertaken to compare plasma testosterone levels in cows carrying male and female foetuses and to study whether abortion was preceded by elevated maternal testosterone. A detailed study of plasma testosterone in periparturient cows was included.

MATERIALS AND METHODS

Pregnant cows of the Norwegian Red Breed were studied. Three to five blood samples were taken from each of 94 cows at 7-day intervals between the 35th and the 125th day of gestation. Eleven cows were slaughtered before term, 14 animals recycled, 35 cows delivered heifer calves and 34 bull calves. One to four blood samples were taken from an additional 164 cows between 125th day of gestation and parturition. A hundred cows delivered bull calves and 64 delivered heifer calves. Twenty-seven cows were monitored closely by taking 4—14 blood samples of each during the days and hours around parturition. Thirteen delivered bull calves and 14 delivered heifer calves.

All blood samples were collected by jugular venipunceture in heparinized vacutainers (Becton Dickinson Ltd., France) and centrifuged. The supernatant plasma was stored at —20°C until analysed. Plasma testosterone was measured by use of radioimmunoassay according to *Sanwal et al.* (1974) as modified by *Sundby et al.* (1975) with a few additional modifications. Testosterone was extracted from 200 µl plasma using 2 ml ether. The relatively high precision of extraction (86.4 ± 4.0 %, n = 45)

allowed the use of a correction factor instead of an internal standard in the assay. Standard solutions of 10, 20, 40, 50, 80, 100, 250 and 500 pg testosterone were assayed for the construction of the standard curve. Fifteen pg tracer and 300 µl of a 1:300,000 dilution of ovine antiserum was used in the assay. The sensitivity of the method was 45 pg. The precision of the method estimated as $s = \pm \sqrt{\frac{\Sigma d^2}{2n}}$ $(n = 355)$ was ± 15.9 pg corresponding to a coefficient of variation of 12 %.

RESULTS

As indicated in Fig. 1 no significant difference was found in plasma testosterone between cows carrying male or female foetuses during the period studied. When the period of gestation was split into 10-days intervals, no significant variation was seen in plasma testosterone in the cows from day 35 to day 80. From day 80 to day 180 plasma testosterone concentration in the cows showed a significant change ($P < 0.01$) and the mean rose from 90—100 pg/ml to 200 pg/ml. From then on levels remained stable until parturition. A significant drop in plasma testosterone concentration occurred the first day after delivery

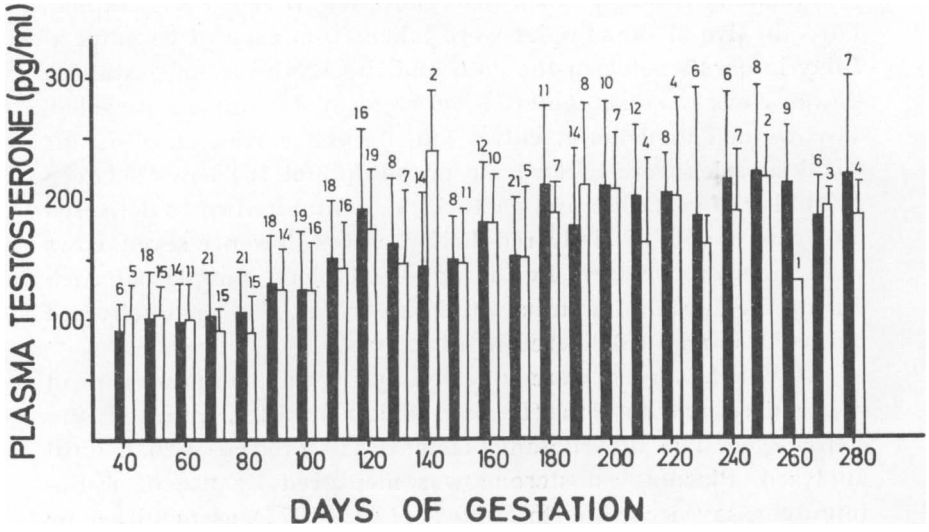

Figure 1. Plasma testosterone in cows with male (■) or female (□) fetus from day 35 to day 280 of gestation. Average + s at 10-day intervals. The number of cows is indicated for each group.

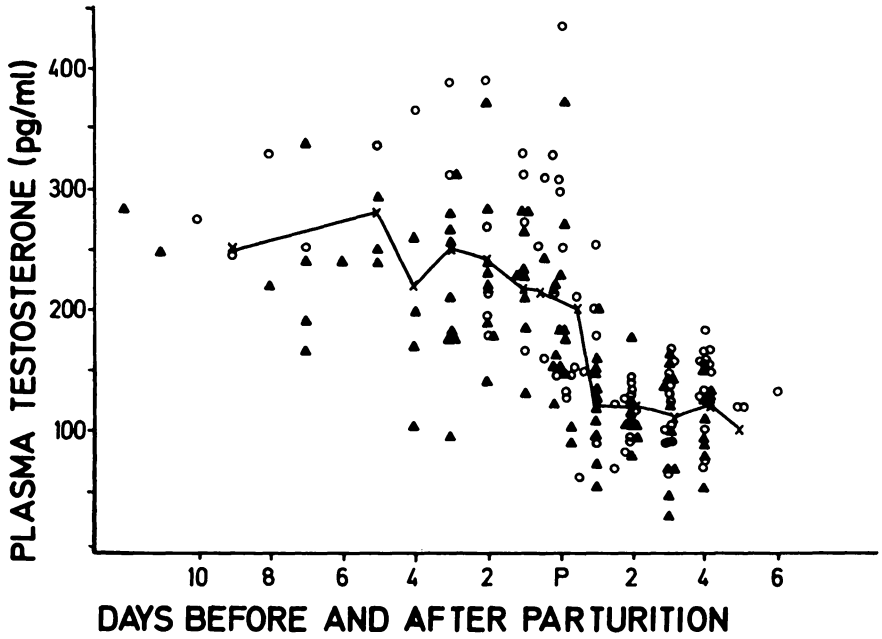

Figure 2. Plasma testosterone in cows with male (▲) or female (○) fetus near parturition.

and this lower level was measured the following 6—10 days (Fig. 2). Among the 14 cows which recycled, only 3 showed higher testosterone than mean + 2 s at any point in time.

DISCUSSION

Differences in plasma testosterone between mothers with male and female foetuses have been found in monkeys (*Resko* 1970), but not in man (*Mizuko et al.* 1968, *Rivarola et al.* 1968). The male bovine foetus is found to have significantly higher testosterone concentration in umbilical cord and arterial blood than the female (*Kim et al.* 1972, *Challis* 1974, *Mongkonpunya et al.* 1975). In spite of this, we found no significant difference in plasma testosterone between pregnant cows carrying male or female foetus at any point in time during gestation. In addition, high maternal testosterone was only observed once at any point in time in 3 of the 14 cows which recycled. The present results are in accordance with the theory of a distinct separation between maternal and foetal steroid circulation in the bovine (*Hoffmann* 1976). Our findings are also in agreement with that

of *Kim et al.*, but different from the observations bade by *Mongkonpunya et al.*, who found that cows carrying male foetuses had significantly higher plasma testosterone in blood samples taken from the uterine and jugular vein on day 90, 180 and 260 of gestation, than those carrying female foetuses. The present investigation indicates that plasma testosterone in cows, irrespective of sex of foetus is kept on a level around 90 pg/ml from day 35 to day 80 of gestation. Thereafter plasma testosterone concentration rises to reach more than double this level (200 pg/ml) on day 180 and remains at this high level until past parturition when a significant decrease occurs within the first day. This profile is in accordance with the results of *Mongkonpunya et al.* (1975) who measured 2—3 times higher plasma testosterone in Holstein heifers at day 180 and day 260 than at day 90 of gestation. In the latter investigation plasma testosterone concentrations were 2 times higher in cows with a male foetus than what we measured in the present study. In pregnant women from 4th—8th week and throughout gestation plasma testosterone levels remained at twice the concentration in non-pregnant women. Then, at delivery, a further rise in plasma testosterone level was observed and, in accordance with the present observation in cattle, a significant fall in plasma testosterone was measured on the first day post partum (*Mizuko et al.* 1968). In the cow, both corpora lutea and ovarian follicles are able to synthesize testosterone in vitro (*Shemesh et al.* 1975, *Mori* 1975). Progesterone is reported to be produced solely by the ovary in pregnant cattle and to be kept on an elevated level of 5—20 ng/ml plasma from the beginning of gestation with a gradual decrease during the last trimester and a rapid fall to 1 ng/ml about 30—40 h before parturition (*Pope et al.* 1969, *Stabenfeldt et al.* 1970, *Randal & Erb* 1971). The main production site for oestrogen is found to be the foetal-placental unit. Free oestrogens in the bovine plasma is kept below 5 pg/ml until late pregnancy. About 14 days before parturition an abrupt increase occurs and the plasma oestrogen concentration reaches 500—2000 pg/ml during the last week before parturition (*Edqvist et al.* 1973, *Convey* 1974). The plasma testosterone profile in the pregnant cow seems to be different from the profile of plasma progesterone and from that of oestrogens. However, the increase in maternal plasma testosterone seems to occur at the time when the urinary oestrogen excretion is starting to increase

(*Velle* 1958, *Randal & Erb* 1971). This is in accordance with the fact that testosterone is an intermediate in oestrogen synthesis. The significant fall in plasma testosterone during the first day after parturition indicates the placenta to be the source of increased testosterone production during gestation. In man, *Cedard et al.* (1963) reported that the capacity of the placenta to convert testosterone to oestrogen in vitro increases with progressing pregnancy. In the pregnant gorilla, *Seaton* (1978) found a significant correlation between oestradiol and testosterone until 11 days before parturition. Furthermore, the rat placenta is found to synthesize testosterone in response to human chorionic gonadotropin (*Chan* 1977).

In conclusion, the present investigation indicates a placental testosterone production from day 80 and throughout gestation and a lack of influence by the foetal sex on maternal plasma testosterone level.

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SAMMENDRAG

Plasma testosteron i kyr i relasjon til drektighetsdag og kjønn av fosteret og plasma testosteron rundt fødsel.

Testosteron ble målt perifert i plasma hos i alt 258 kyr, fra dag 35 i drektigheten og frem til partus. Resultatene ble sammenstilt for hvert 10-dagers intervall og verdiene sammenlignet. Ikke på noe tidspunkt ble det påvist signifikante forskjeller mellom kyr med hannfoster og hunnfoster. Den rapporterte høyere abortfrekvens for hannfostre enn for hunnfostre synes derfor ikke å kunne tilskrives effekten av testosteron secernert fra den føtale testis på mordyrets endokrine balanse.

Til tross for store individuelle variasjoner i testosteronkonsentrasjonen i plasma på samme tidspunkt i drektigheten, ble det påvist en tydelig økning utover i drektigheten. I perioden 35—80 dager lå nivået på 80—100 pg/ml. Nivået steg så frem til 180. dag hvoretter det stabiliserte seg på ca 200 pg/ml frem til fødselen. Etter fødselen sank nivået i løpet av første dag og stabiliserte seg på 120 pg/ml.

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