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Dehydroepiandrosterone and Epitestosterone in the Blood of Cows at Term

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With 3 figures

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Summary

From Day 268 of gestation till two days after parturition blood samples were collected from the jugular vein of 5 cows with normal and 4 cows with flumethasone induced parturition and centrifuged immediately after sampling.

In another experiment blood samples were taken from the jugular and uterine vein of 3 cows during the last week before normal parturition. The dehydroepiandrosterone (DHA) and epitestosterone (ET) concentrations in plasma were measured using radioimmunoassay.

In the control group the DHA concentration was in the range of 2 to 5 nmol/l, ET levels varied from 4 to 8 nmol/l before term. In both groups, there was an increase in DHA concentrations during the last periparturient period whereas ET levels increased only after flumethasone induced parturition. The concentrations of both androgens declined after the expulsion of the placenta and they were higher in the uterine vein than in the jugular vein.

It is concluded, that both androgens are secreted by the bovine placenta and that the △ 5-pathway of steroidogenesis is active *in vivo*.

Key words: Cattle, parturition, dehydroepiandrosterone, epitestosterone, androgens

Introduction

The concentration of oestrogen in bovine plasma increases during the last weeks of gestation. The dominant oestrogen is oestrone (Dobson and Dean, 1974; Robertson, 1974), which is secreted into the maternal compartment mainly in the conjugated form (Hoffmann et al., 1979).

In vitro, the bovine placenta synthesizes free and conjugated oestrogens from various androgens (Ainsworth and Ryan, 1966; Pierrepoint et al., 1969; Evans and Wagner, 1981; Möstl et al., 1987) but there are only few reports suggesting that androgens appear in the peripheral blood of cows at the end of gestation (Mongkonpunya et al., 1975; Möstl et al., 1981).

During the last week of gestation, circulating concentrations of cortisol increase in the bovine fetus (Comline et al., 1974). In the presence of high amounts of glucocorticoids the

activity of the placental enzyme system involved in androgen biosynthesis in the goat (and apparently in the cow) is enhanced *in vitro* (Flint et al., 1979). In sheep, androstenedione is elevated at the end of gestation (Steele et al., 1976), whereas the concentration of this androgen in cows increases only after administration of a synthetic glucocorticoid (Möstl et al., 1985).

Androgens for subsequent aromatization can be produced from pregnenolone by the △ 4- or △ 5-pathways. *In vitro* culture with late gestation bovine placental samples yields higher conversions from pregnenolone rather than 17 α-hydroxyprogesterone into oestrogens, indicating that the △ 5-pathway is more active (Evans and Wagner, 1981).

The bovine placenta does not need fetal precursors, as removal of the fetus by preterm caesarian section did not terminate oestrogen production during the period of retained fetal membranes (Hoffmann et al., 1979).

The objective of this investigation was to determine whether the bovine placenta was secreting androgens (e. g. dehydroepiandrosterone) during the pre-term period. Positive results would indicate that the oestrogen precursors were produced by the placenta itself.

As dehydroepiandrosterone (DHA) and androstenedione are the key intermediates of oestrogen biosynthesis (Gower and Fotherby, 1975), we measured the concentration of these two androgens in peripheral blood of cows during the periparturient period as a parameter of androgen biosynthesis.

As androstenedione is quantitatively converted by bovine red blood cells into epitestosterone (Lindner, 1961; Möstl et al., 1981), the epitestosterone (ET) concentration in the peripheral blood was measured instead of androstenedione.

Material and Methods

In 5 cows with spontaneous parturition around Day 290 and 4 cows with flumethasone (3.5 mg/animal; i. m.) induced parturition on Day 272 of gestation the androgen concentrations in the peripheral plasma were measured. Blood samples were collected every 3 hours from the jugular vein into heparinized tubes from Day 268 of gestation until 2 days after parturition.

In another 3 pregnant cows (between Day 260 and 280 of gestation) blood samples were collected 6 times during a period of 48 hours from the jugular vein and the uterine vein.

All samples were centrifuged immediately and the plasma stored at − 20 °C until analysis to avoid the *in vitro* conversion of DHA.

The DHA and ET radioimmunoassays were performed as previously described (Möstl et al., 1981; Choi, 1987). The antibodies were raised in rabbits using 5-androstene-3 β-ol-7,17-dione-7-CMO : BSA (DHA) or 4-androstene-17 αol-3one-CMO : BSA (ET). ^{3}H-DHA was purchased from New England Nuclear, Dreieich, FRG (NET 479), ^{3}H-ET was prepared as described earlier (Möstl et al., 1983).

The antiserum for DHA showed crossreactions with 4-androstene-3,17-dione (5.9 %) and 5-androstene-3 β,17 β-diol (1 %). The crossreactions of both antisera with other relevant steroids tested were less than 0.1 %.

The average recovery after extraction was 87.5 % (DHA) and 84.3 % (ET). The within and between assay coefficients of variation were 6 and 12 % for DHA, 9 and 14 % for ET.

In the group with spontaneous parturition only two samples per day were analyzed from 21 days to 3 days before parturition. In both groups analysis was performed on all samples collected between 48 h pre- and 24 h post-parturition.

In the control group mean values and standard errors were calculated. In the group with induction of parturition the individual values are shown because there were only four animals in this group and high variations between individuals were measured.

Results

The concentrations of DHA and ET in the blood of cows around spontaneous parturition are shown in the figure 1.

In the control group the concentration of DHA was in the range of 2—5 nmol/l. The ET concentrations were somewhat higher (4—8 nmol/l) than the DHA concentrations and remained almost constant during the pre-term period. The ET values decreased gradually

after parturition. In contrast DHA concentration increased until three hours after parturition and decreased to basal values within 6—12 h. Basal values of ET were also found by this time (Fig. 1).

After induction of parturition with flumethasone there was an increase in DHA and ET in the plasma of the dams (Figs. 2 and 3). One cow showed very high pre-term ET values, reaching about 50 nmol/l (Fig. 3). This cow had retained fetal membranes. After 24 hours the concentrations of both androgens declined to basal levels. Three animals of this group had retained fetal membranes for longer than 12 hours.

In the uterine vein the amount of DHA and ET was higher than in the jugular vein. The differences were more pronounced in ET than in DHA values (*V. jugularis:* ET 8,6 ± 2,38 nmol/l, DHA 3,6 ± 0,60 nmol/l; *V. uterina:* ET 19,0 ± 5,78 nmol/l, DHA 4,76 ± 1,36 nmol/l).

Discussion

The ET concentrations in the jugular vein were in the same range as described earlier (MÖSTL et al., 1981). Since DHA and ET were present in the uterine vein in higher amounts than in the jugular vein, both androgens are produced by the bovine placenta or by the fetus.

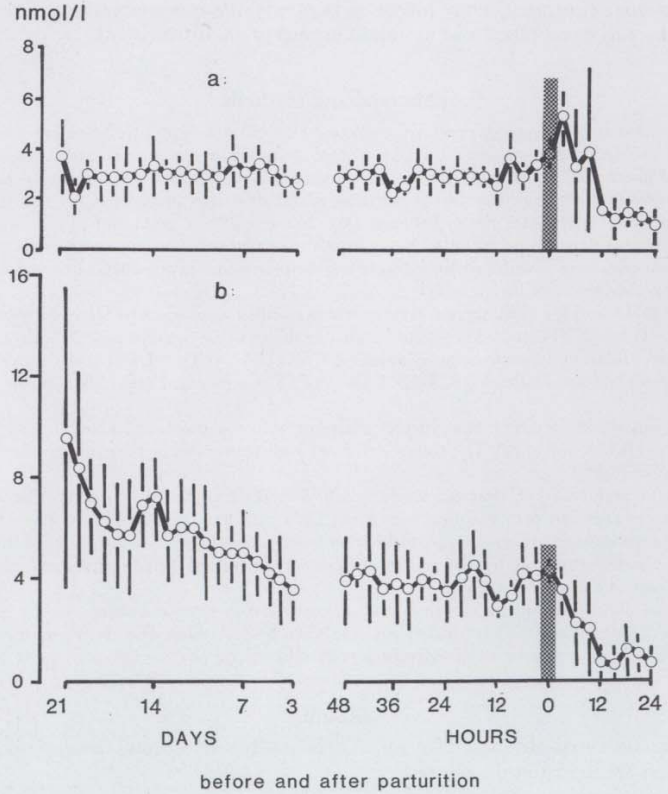


Fig. 1. Concentration (mean ± SEM; n = 5) of (a:) DHA and (b:) ET in plasma of cows before and after spontaneous parturition (▦ time of parturition)

Fig. 2. Concentration of DHA in plasma of cows before and after glucocorticoid-induced parturition (▦ time of parturition)

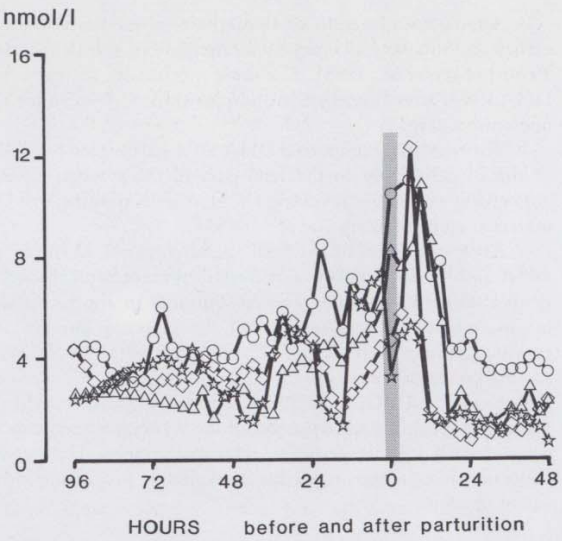
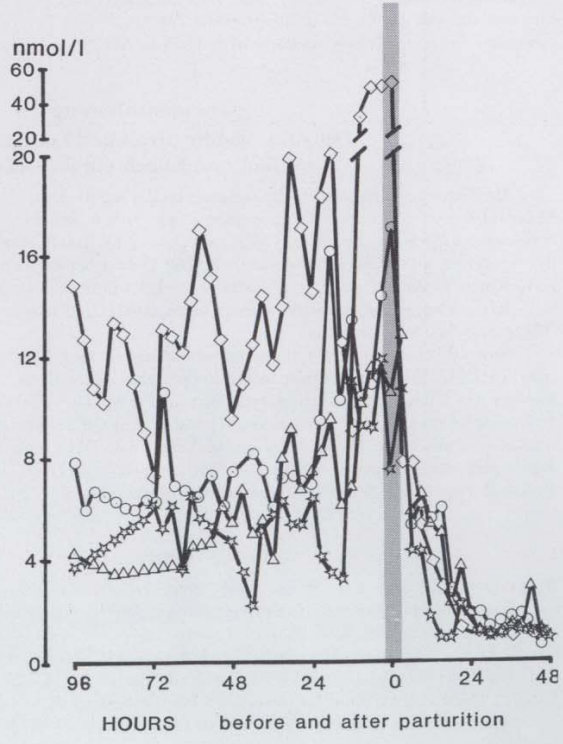


Fig. 3. Concentration of ET in plasma of cows before and after glucocorticoid induced parturition (▦ time of parturition)



After administration of flumethasone, increased ET concentrations were described earlier and can be explained by an increase of the 17 α-hydroxylase activity (FLINT et al., 1979; MÖSTL et al., 1985). The same mechanism seems to be the reason for the increased DHA levels after flumethasone administration. We cannot explain the high ET increase in one animal (Fig. 3).

The transient increase of DHA after parturition may be explained by the termination of the blood supply to the fetal part of the placentome and inactivation of the DHA-converting enzymes, resulting in increased amounts of DHA being excreted into the maternal compartment.

After expulsion of the fetal membranes (< 12 hours after spontaneous parturition) DHA and ET concentrations were still present in the blood of cows. This could represent gradual absorption from residual amounts in the lochia or mean that these androgens originate from sources other than the placenta. Bovine ovaries or adrenal glands may produce androgens (CHOI et al., 1983; SIMONIAN et al., 1979) and possibly contribute to plasma concentrations.

EVANS and WAGNER (1981) reported a preference for the △ 5-pathway of *in vitro* oestrogen synthesis since pregnenolone was converted more efficiently than 17 α-hydroxy-progesterone by late gestation fetal membranes. The demonstration of increased DHA concentrations in uterine plasma immediately prepartum indicated that this pathway is also active *in vivo*.

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Zusammenfassung

Dehydroepiandrosteron und Epitestosteron im Blut von Kühen um die Geburt

Im Zeitraum vom 268. Trächtigkeitstag bis 2 Tage nach der Geburt wurden Blutproben aus der *V. jugularis* von 5 Kühen mit spontaner sowie von 4 Kühen mit flumetasoninduzierter Geburt entnommen. Bei weiteren 3 Tieren wurden während der letzten Woche der Gravidität Blutproben aus der *V. uterina* und *V. jugularis* gesammelt. Die Dehydroepiandrosteron(DHA)- und Epitestosteron (ET)-Konzentrationen im Plasma wurden mittels Radioimmunoassay bestimmt.

In der Gruppe mit spontaner Geburt lagen die DHA-Werte im Bereich von 2—5 nmol/l, die ET-Werte zwischen 4—8 nmol/l.

Sowohl bei den Tieren mit spontaner Geburt als auch bei den Kühen mit induzierter Geburt stieg der DHA-Gehalt im unmittelbaren peripartalen Zeitraum an, während die ET-Konzentrationen nur bei den Tieren mit induzierter Geburt anstiegen. Die Werte beider Androgene waren in der *V. uterina* höher als in der *V. jugularis*. Nach dem Abgang der Plazenta sanken die Androgenkonzentrationen deutlich ab. Es ist daher anzunehmen, daß DHA und ET (als Androstendion) von der Rinderplazenta gebildet werden. Das Vorkommen von DHA ist ein Indiz dafür, daß in der Rinderplazenta der 5-Weg der Steroidhormonbiosynthese aktiv ist.

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