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Free and Conjugated Steroids in Maternal and Fetal Plasma in the Cow Near Term

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ABSTRACT

Plasma samples were collected in 5 pregnant cows (between Days 247 and 273 postconception) via indwelling catheters from the maternal jugular and uterine veins, umbilical artery and vein and the fetal vena cava caudalis. Four fetuses died within four days and the fifth was born alive on Day 3 postsurgery. Progesterone (P), Testosterone (T) as well as free and conjugated (enzyme hydrolysis) cortisol (F), corticosterone (B), estrone (E_1), estradiol-17α and -17β ($E_{2\alpha}$, $E_{2\beta}$) were determined by CPB and RIA respectively. Steroid concentrations showed a distinct separation between maternal and fetal circulation. Fetal P was low ($\bar{x}$ = 0.20 ng/ml) but maternal P was high as anticipated for the stage of pregnancy, while T values were low in all sampling sites (0.08 – 0.55 ng/ml). Corticoids were higher in the maternal than fetal compartment except in one cow which was cannulated post Day 270 where F increased in the fetus within 4 days from 71 to 102 ng/ml on the day of parturition. No significant changes in F/B-ratios occurred in maternal samples while fetal plasma F/B ratios increased during the sampling period. Conjugated F and B were 5 percent and 10 – 20 percent of the free, respectively, while conjugated estrogens could exceed the free estrogens by a factor of 10 to 100 reaching concentrations above 100 ng $E_{2\alpha}$/ml in 2 samples in the fetus. Free and conjugated E_1 was the major maternal estrogen while it was $E_{2\alpha}$ in the fetus. In both compartments lowest concentrations were found for $E_{2\beta}$ (free: 0.007 – 0.060 ng/ml, conjugated 0.04 – 1.69 ng/ml, depending on the sampling site). Except for $E_{2\beta}$ and based on paired samples, estrogen levels in the uterine vein were higher than in the jugular vein, and in the fetus they were highest in the vena cava and lowest in the umbilical artery.

INTRODUCTION

Previous studies in the pregnant cow have clearly shown that, to obtain more information on hormonal control of pregnancy and parturition, studies have to be extended from the mother to the fetus. In the present paper results are given of blood levels of several steroids in different sampling sites in the mother and fetus, using simultaneous sampling from indwelling catheters.

MATERIALS AND METHODS

Animals and Blood Sampling

Four Simmental and one Holstein cow were cannulated between Days 247 to 270 postconception. Five silastic catheters were implanted. In the fetus these were the umbilical vein and artery and posterior vena cava via the lateral saphenous vein. The maternal system was sampled from catheters in the jugular and uterine veins. A combination of general sedation with a tranquilizer (Rompun, Fa. Bayer), a short acting barbiturate (Thiogenal, Fa. Bayer) and local anesthesia (Tutocain, Fa. Bayer) was used during surgery. Blood samples (5–10 ml) were collected once daily at 0800 in heparinized syringes. After cooling, the blood was immediately centrifuged and the plasma was stored at −20°C until assayed.

Hormone Assay

Free steroids: Progesterone, corticosterone, cortisol

From each plasma sample two 200 µl aliquots were removed. After a first extraction with petroleum ether, progesterone was determined by radioimmunoassay (RIA) (Hoffmann et al., 1973a). Corticoids were assayed by a competitive protein binding technique using a second extraction of the same plasma aliquots with dichloromethane (Hoffmann et al., 1973b). The separation of cortisol from corticosterone was achieved by chromatography on Sephadex-LH 20 microcolumns applying the solvent system of dichloromethane/methanol (98/2) (Murphy, 1971). Average recoveries for these steroids were progesterone (95 percent), cortisol (72 percent ± 8.2) and corticosterone (85 percent ± 7.5).

Testosterone

For testosterone assay, two aliquots of 200 µl were taken from each plasma sample and quantitation was

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performed by RIA as previously described (Karg et al., 1976). Recovery of testosterone was demonstrated to be constant at about 92 percent.

Estrone (E_1), Estradiol-17β ($E_{2\beta}$), Estradiol-17α ($E_{2\alpha}$)

For these RIA, highly specific antisera directed against the individual estrogen-C6-conjugates were used. Binding characteristics and cross-reactivity followed the specifications outlined by Exley et al. (1971), Kuss and Goebel (1972a,b) and Kuss et al. (1973). Five hundred μl of plasma in duplicate were used for extraction. The procedure is outlined in detail elsewhere (Hoffmann, 1972) and includes a separation of estrone from estradiol (17α and 17β) on Sephadex-LH 20 columns (benzene/methanol – 85/15). The fraction containing the estradiol was split into 2 parts, one serving for the measurement of estradiol-17β and one for the determination of estradiol-17α. In addition to the specificity of the antisera used (directed against C-6 – conjugated $E_{2\beta}$ and $E_{2\alpha}$ – see above) this procedural step was further validated by measuring in the identical plasma samples $E_{2\alpha}$ before and after separation from $E_{2\beta}$, applying a Bush-type paper-chromatography (overrun for 15 h in the system isooctane/toluene (2:1) vs. 80 percent methanol). The values obtained were not different with a correlation of r = 0.995 and a coefficient of variation of 7.8 percent. Depending on the sampling-site (fetal plasma, uterine vein; see results) the eluates containing the estrone and estradiol-17α had to be further diluted and aliquots then removed for RIA. Percentage recovery of free estrogens was E_1 (87.0 ± 4.3), $E_{2\alpha}$ (84.6 ± 6.0) and $E_{2\beta}$ (81.7 ± 5.5).

Conjugated Steroids

Corticosterone, cortisol, estrone, estradiol-17β and 17α were determined in plasma samples after extraction of the free steroids and following enzyme hydrolysis (Jayle et al., 1959; Gomes et al., 1965) using a glucuronidase-arylsulfatase preparation (β-Glucuronidase Arylsulfatase, Helix pomatia, Boehringer Mannheim) (Graef and Fuchs, 1975). Hydrolysis was performed by adding 1.5 ml acetic buffer (pH 4.8, 0.2 molar) and 5 μl enzyme preparation (diluted 1:10 with acetic buffer) to each plasma sample. The samples were then incubated for 1 h at 37°C. Control experiments with estradiol-6, 7-^{3}H-17β-D-glucuronide (50 Ci/mM, New England Nuclear) gave an average recovery of 86.7 ± 5.5 percent free estradiol-17β. Following hydrolysis internal standards were added and all samples were extracted once with anesthetic grade ether (6 ml). The extracts were then washed with 1.0 ml phosphate buffer and with 2 times 2 ml distilled water and evaporated. The procedure thereafter was identical with the procedure for the free steroids. Results were only corrected for procedural losses following hydrolysis. The recovery data were 91.9 ± 4.3 for E_1, 90.1 ± 6.6 for $E_{2\beta}$ and 89.4 ± 6.2 percent for $E_{2\alpha}$.

RESULTS

Surgery

One cow calved on Day 3 after surgery (surgery = Day 0) with a live calf. In the other four animals the fetus died; in one cow 2 days after surgery and in the other 3 animals 4 days after surgery. Thus no samples were collected longer than 4 days. The umbilical artery was a particular problem since we were not able to obtain blood from this cannula longer than 1 or 2 days in most animals.

Hormone Determinations

Progesterone. Jugular plasma progesterone values (ng/ml, $\bar{x}$ ± S.E.) were 7.88 ± 0.60 for all five cows compared to a value of 10.63 ± 1.73 for the uterine vein samples. However, uterine vein levels were very high in one animal where the tip of the cannula was apparently lying proximal to the ovary. With this animal removed, uterine vein values averaged only 5.40 ng/ml. In the cow which calved, progesterone values in jugular vein plasma declined from 8.5 ng/ml on the day of surgery to 1.1 ng/ml on the day of parturition. Progesterone concentrations were very low in fetal plasma, averaging 0.20 ng/ml for all sampling sites. Although there were slight differences between the three sampling sites in the fetus no significant trends were observed.

Testosterone. Testosterone values were generally low in both maternal and fetal blood averaging 0.33, 0.55, 0.08, 0.39 and 0.19 ng/ml for the jugular and uterine vein, umbilical artery and vein and fetal vena cava, respectively.

Corticoids. The corticoid data are presented for the four animals sampled before 270 days of pregnancy in Table 1 and for the animal that calved (>270 days) in Table 2. In Table 1 it is evident that cortisol (F) was higher in maternal plasma. The maternal values were very high on the day of surgery and declined thereafter. Corticosterone (B) apparently decreased parallel to cortisol since the maternal F/B ratio did not change significantly. Fetal corticoids showed no immediate response on the day of surgery, but gradually increased from 9.9 to 16.7 ng/ml over the sampling period. The ratio F/B also differed between maternal and fetal individuals with the ratio increasing in fetal plasma as cortisol values increased suggesting that corticosterone does not change in connection with cortisol in the fetus as it appeared to do in the mother. Table 2 presents even more clearly the difference between maternal and fetal compartments since maternal cortisol decreased during the sampling period while fetal

TABLE 1. Plasma corticoids (mean ± S.E.M.) in bovine maternal[a] and fetal[b] plasma. Samples collected before day 270 of pregnancy.

Days post-surgery	Cortisol (F)				F/B ratio[c]	
	Maternal		Fetal			
	No. samples	ng/ml	No. samples	ng/ml	Maternal	Fetal
0	8	98.3 ± 11.0	8	8.8 ± 1.5	7.8	10.4
1	7	43.1 ± 13.8	7	11.0 ± 1.9	8.8	16.3
2	5	12.3 ± 1.4	6	12.8 ± 2.6	6.0	65.1
3	5	16.8 ± 3.8	3	16.7 ± 0.5	10.5	71.3

[a]Mean of jugular and uterine vein.

[b]Mean of umbilical vessels and vena cava.

[c]F represents cortisol and B represents corticosterone.

cortisol steadily increased to the day of parturition. The change in F/B ratio was even more striking in this fetus since corticosterone levels slightly decreased while the cortisol level was rising. Conjugated corticoids in maternal and fetal plasma were found to be quite low in most samples. The amounts of conjugated cortisol were generally less than 5 percent of free cortisol while conjugated corticosterone represented about 10 – 20 percent of the amount of free corticosterone.

Estrogens. Table 3 presents the data for free estrogens. Both E_1 and $E_{2\alpha}$ were higher in the uterine vein than in the jugular vein while $E_{2\beta}$ was higher in the jugular vein. These differences were especially apparent in the animal pregnant over 270 days with respect to E_1. In this animal uterine vein blood levels of E_1 rose significantly from 1500 pg/ml on the day of surgery to 6100 pg/ml 3 days later (day of parturition) while jugular levels remained constant. In fetal plasma the highest levels of free estrogens occurred in the vena cava and the lowest in the umbilical artery with $E_{2\alpha}$ as the major estrogen.

In contrast to the free estrogens, there were very large concentrations of conjugated estrogens in both maternal and fetal plasma samples (Table 4). In all samples $E_{2\beta}$ was present in rather small amounts compared to E_1 and $E_{2\alpha}$. In maternal plasma E_1 was the predominant estrogen in both uterine and jugular plasma. In the cow which calved, conjugated E_1 levels averaged 27.3 ng/ml in uterine vein plasma and were higher than in jugular plasma (17.8 ng/ml). Conjugated estrogen levels were even higher in the fetal samples where $E_{2\alpha}$ was the predominant estrogen. Levels were again highest in the fetal vena cava and lowest in the umbilical artery. When values for E_1 and $E_{2\alpha}$ are compared in umbilical artery and vein plasma for 5 paired samples (Table 5), it is evident that the fetus is removing substantial quantities of con-

TABLE 2. Plasma corticoids (ng/ml) in bovine maternal[a] and fetal[b] plasma from a cow cannulated on day 270 post conception.

Days post-surgery	Cortisol (F)		F/B[c] ratio	
	Maternal	Fetal	Maternal	Fetal
0	90.0	71.0	4.5	35.8
1	11.1	77.2	8.5	77.2
2	10.4	95.6	8.7	81.1
3	3.8	102.1	3.8	136.1

[a]Mean of jugular and uterine vein.

[b]Mean of umbilical vessels and vena cava.

[c]F represents cortisol and B represents corticosterone.

TABLE 3. Free estrogens (mean ± S.E.M.) in bovine maternal and fetal plasma expressed as pg/ml plasma.

Site	<270 days pregnant				>270 days pregnant			
	No. samples	Estrone	Estradiol-17α	Estradiol-17β	No. samples	Estrone	Estradiol-17α	Estradiol-17β
Jugular vein	15	584 ± 56	91 ± 21	26 ± 5	4	1425 ± 81	101 ± 12	60 ± 11
Uterine vein	11	652 ± 116	154 ± 42	7 ± 1	4	4121 ± 707	197 ± 16	36 ± 19
Umbilical artery	5	85 ± 34	223 ± 79	7 ± 5	1	77	254	8
Umbilical vein	8	103 ± 32	201 ± 25	9 ± 3	4	267 ± 78	662 ± 338	9 ± 5
Fetal vena cava	13	179 ± 49	881 ± 423	38 ± 22	a			

[a]No plasma samples obtained.

TABLE 4. Conjugated estrogens (mean ± S.E.M.) in bovine maternal and fetal plasma, expressed as ng/ml plasma.

Site	<270 days pregnant				>270 days pregnant			
	No. samples	Estrone	Estradiol-17α	Estradiol-17β	No. samples	Estrone	Estradiol-17α	Estradiol-17β
Jugular vein	15	11.32 ± 2.12	3.01 ± 0.34	0.11 ± 0.03	4	17.85 ± 0.47	4.94 ± 0.38	0.42 ± 0.11
Uterine vein	11	7.95 ± 2.16	2.32 ± 0.63	0.04 ± 0.01	4	27.33 ± 2.66	4.70 ± 0.69	0.37 ± 0.09
Umbilical artery	5	13.63 ± 6.51	36.85 ± 13.92	0.76 ± 0.31	1	3.69	15.41	a
Umbilical vein	6	14.60 ± 1.88	42.33 ± 3.14	0.37 ± 0.09	4	45.98 ± 10.01	89.02 ± 10.52	0.39 ± 0.03
Vena cava	13	25.47 ± 5.97	75.20 ± 23.52	1.69 ± 0.69	b			

[a]Not assayed.

[b]No plasma sample obtained.

TABLE 5. Estrogens (ng/ml) in umbilical circulation; values based on 5 paired samples from 4 animals.

	Estrone		Estradiol-17α	
	Umbilical artery	Umbilical vein	Umbilical artery	Umbilical vein
Free	0.041	0.180	0.080	0.190
Conjugated	2.825	13.784	11.952	43.675

jugated estrogens from the blood. The highest mean values occurred in the fetus that was born. In two consecutive samples, $E_{2\alpha}$ concentrations exceeded 100 ng/ml just prior to parturition. The distinct difference in estrogen secretion between the mother and fetus can be seen from Table 6. A comparison of the concentrations of E_1 and $E_{2\alpha}$ in uterine and umbilical vein plasma based on 11 paired samples indicate the reversal of $E_1/E_{2\alpha}$ ratios between maternal and fetal compartments again demonstrating that E_1 is the major maternal estrogen, while $E_{2\alpha}$ is the major fetal estrogen in both the free and conjugated form.

DISCUSSION

Although difficulties with fetal survival occurred which may have altered some specific hormonal secretion rates, we believe that the comparisons between sampling sites are still valid. The low progesterone concentrations found in all fetal plasma samples agree with the data of Challis et al. (1974). In this respect the bovine fetus is much different from the ovine fetus (Thompson and Wagner, 1974; Basset and Thorburn, 1973) as well as the porcine fetus (Barnes et al., 1974; Godke and Day, 1973) where higher levels are found in the fetal than in the maternal compartment. The species differences may be related to placental permeability, although it is difficult to explain placental transfer of progesterone to the porcine fetus (Barnes et al., 1974) and not in the cow. The relatively high concentrations in the ovine fetus may result from the placental progesterone production which occurs in the sheep (Short and Moore, 1959). Maternal progesterone values seen in this study confirm previous reports that uterine vein progesterone concentration is lower than jugular plasma (Comline et al., 1974; Wagner et al., 1974; Evans and Wagner, 1976). In one animal this trend was reversed, apparently due to incorrect placement of the catheter so that ovarian drainage was being collected. In this cow uterine plasma progesterone ranged from 17 – 30 ng/ml which would agree with the values for utero-ovarian plasma as reported by Evans and Wagner (1976). Thus in addition to confirmation of previous data that uterine vein plasma does not make a net contribution to circulating levels of progesterone (Wagner et al., 1974), these data further suggest there is no significant secretion of progesterone from or via the placenta into the fetal compartment.

Testosterone levels found in this study agree with previously published data for this stage of pregnancy (Challis et al., 1974). Due to the small number of animals no attempt was made to differentiate between male and female fetuses. Certainly other androgenic steroids, especially dehydroepiandrosterone or its sulfate need to be measured in similar studies, especially in regard to their function as androgenic precursors for estrogen production (see Ainsworth and Ryan, 1966; Lamb et al., 1967).

TABLE 6. Comparison of estrogens (ng/ml) in maternal and fetal circulation; values based on 11 paired samples from 5 animals.

	Maternal (Uterine vein)			Fetal (Umbilical vein)		
	E_1	$E_{2\alpha}$	$E_1/E_{2\alpha}$	$E_1/E_{2\alpha}$	E_1	$E_{2\alpha}$
Free	2.105	0.102	20.64	0.39	0.158	0.396
Conjugated	16.042	2.806	5.72	0.45	28.409	62.371

Measurement of cortisol and corticosterone has confirmed that corticoids increase at parturition in the bovine fetus (Comline et al., 1974; Fairclough et al., 1975). It is also very evident that cortisol (F) increases rather rapidly with no similar increase in corticosterone (B) as evidenced by the marked changes in F/B-ratios.

Although the fetal corticoid values showed no increase on the day of surgery comparable to that seen in maternal plasma, there was a gradual increase over the 3–4 days of sampling. This may be similar to the response seen by Comline et al. (1974), who reported only a gradual increase in corticoid secretion when ACTH was infused into the fetus. Furthermore, very little transfer of cortisol between maternal and fetal compartments seems to occur in the cow, especially when the data in Table 2 are considered where the plasma levels of cortisol in the mother and fetus are moving in opposite directions during the sampling period. From the data presented it is evident that the bovine fetal adrenal can respond by day 250 and produce significant amounts of cortisol. However, 4 to 6 times higher levels of cortisol, up to 102 ng/ml, were observed in the one fetus just prior to birth. Still, these data do not yet definitely answer the question concerning the role of the fetal adrenal in bovine parturition (Adams and Wagner, 1970; Hoffmann et al., 1973b; Comline et al., 1974). In view of the report by Wise et al. (1975), the concentrations of deoxycorticosterone also should be monitored in any further studies.

The importance of specific estrogens during pregnancy and parturition in domestic animals, especially the cow, has not been well characterized. Diczfalusy (1962, 1964) has clearly described the function of the fetoplacental unit as it involves estrogen synthesis. In the present study it is apparent that the bovine placenta is capable of producing rather large quantities of estrogens, most of which are apparently conjugated at the site of synthesis in the placenta. As reported by Evans and Wagner (1976) and Peterson et al. (1975), levels of E_1, the main estrogen found in the mother, were slightly higher in the uterine vein compared to the jugular vein. As was observed earlier by Wagner et al. (1974), in these studies a dramatic rise of free E_1 in the uterine vein was not reflected by levels of free E_1 in the jugular vein in the animal which calved (>270d), raising the question of the physiological importance of peripheral blood plasma levels. The same tendency as for E_1 was true for $E_{2\alpha}$ while $E_{2\beta}$ values demonstrated an opposite trend (see Table 3). Peterson et al. (1975) reported free E_1 and $E_{2\beta}$ to be in the range of 15–25 pg/ml in the fetus. Our data agree with respect to $E_{2\beta}$ while our E_1 values were somewhat higher. Moreover, concentrations of free $E_{2\alpha}$ were still higher, averaging about three times the amount of E_1 and making it the dominant estrogen in the fetal compartment whether <270 or >270 days pregnant. Exceeding the concentrations of the free estrogens by a factor of 10 to more than 100, the conjugated estrogens were significantly higher in both the maternal and fetal compartment during all stages of pregnancy investigated. There is no evidence in these data that there are significant quantities of $E_{2\beta}$, either conjugated or free, present in the fetus. This is in disagreement with the data of Hunter et al. (1974) who reported estrogen levels (free plus conjugated) of E_1: 40 ng/ml, $E_{2\alpha}$: 24 ng/ml and $E_{2\beta}$: 46 ng/ml in samples collected from the posterior vena cava in bovine fetuses. The total of E_2 ($\alpha + \beta$) was not significantly different from that reported here although the relative amounts of $E_{2\alpha}$ and $E_{2\beta}$ were quite different.

The estrogen data obtained during this study also provide clarification of the sites of estrogen synthesis and its transport in the pregnant cow. Both the actual amounts and ratios between E_1 and $E_{2\alpha}$ varied between the maternal and fetal compartments. Since conjugated E_1 and $E_{2\alpha}$ were present in higher levels in the umbilical vein (Table 5) compared to the umbilical artery, the fetus must be capable of metabolizing or excreting rather large quantities of these steroids. Also since the $E_1/E_{2\alpha}$ ratio was <1.0 in the fetus but much greater in the mother (Table 6), it seems unlikely that the estrogens in the maternal blood result from passage of steroids across the placenta from the fetus. Rather the data suggest that each compartment (maternal and fetal) is separate and distinct.

What physiologic process could account for these clearly different estrogen patterns in fetal and maternal plasma? Due to the fact that these different patterns are evident in placental venous effluent, i.e. umbilical and uterine veins, it must originate in the placenta itself. Because of the marked differences in E_1 and $E_{2\alpha}$ content between umbilical artery and uterine vein, it seems unlikely that differences in transport alone could account for these observations. This hypothesis is supported by the fact that

conjugated E_1 is only about twice as high in the umbilical artery compared to the uterine vein (13.63 ng/ml vs 7.95 ng/ml) while conjugated $E_{2\alpha}$ is about 15 times higher in the umbilical artery (36.85 ng/ml) compared to the uterine vein (2.32 ng/ml).

Thus we are left with the possibility that as the placenta synthesizes estrogens it regulates the type and amount of estrogens secreted into each compartment. Since there is much more fetal placenta than maternal placenta in the cow, it is possible that the fetal placenta secretes its synthesized products into the fetal circulation while the maternal placenta secretes its products into the maternal system.

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