Clinical Endocrinology (1979) **11**, 603–610.

INTERRELATIONS BETWEEN PLASMA AND TISSUE CONCENTRATIONS OF 17β-OESTRADIOL AND PROGESTERONE DURING HUMAN PREGNANCY

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(Received 29 January 1979; revised 18 May 1979; accepted 30 May 1979)

SUMMARY

Oestradiol and progesterone concentration in plasma, decidua, myometrium and placenta obtained from women undergoing Caesarian section at term and abortion at weeks 16–22 of pregnancy were determined. There was a significant increase in oestradiol concentration (per g wet wt) both in placenta, decidua and myometrium from mid-term to term. Both at mid-term and term oestradiol concentrations in decidua and myometrium were much smaller than those in the plasma (per ml). Progesterone concentration in placenta and in myometrium did not increase from mid-term to term where it increased significantly in decidua. Decidual and myometrial progesterone concentrations at mid-term were 2–3 times higher than those in plasma, but at term the concentrations in both these tissues were lower than in plasma. The ratio progesterone/oestradiol in plasma, decidua, myometrium and placenta at mid-term was 8·7, 112·2, 61·4 and 370·0, respectively, and it decreased significantly in the myometrium and placenta but was nearly unchanged in plasma and decidua at term. The general conclusion to be drawn from the present study is the lack of correspondence between the plasma concentrations and the tissue concentrations of female sex steroids during pregnancy.

We have previously shown that at term, the concentration of 17β-oestradiol and progesterone in the myometrium is not related to the plasma concentrations of these steroids and the myometrium contains much less oestradiol than progesterone (Batra & Bengtsson, 1978). The myometrium/plasma ratio for oestradiol at the end of pregnancy was less than 0·2.

In several recent theories concerning mechanisms for the initiation of human parturition, decidual oestradiol and progesterone was assigned a key role in the production of prostaglandins, which in turn led to the expulsive myometrial contractions (Liggins, 1974; Gustavii, 1975; Challis *et al.*, 1974). Oestrogens and progesterone were assumed to control the stability of decidual lysosomes (Schwarz *et al.*, 1975; Sykes *et al.*, 1975; Keirse

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0300-0664/79/1200-0603$02.00

& Turnbull, 1976). We wondered, therefore, whether the concentrations of these steroids in the decidua were related to those in the plasma and/or those in the myometrium, and if so in what manner.

We have therefore measured the concentration of oestradiol and progesterone in the plasma, myometrium, decidua and placenta of pregnant women at mid- and full term. The data were analysed in terms of the interrelationships between the concentration of either steroid in the various tissues and in plasma, as well as the extent to which these relations were altered from mid-term to the full term of pregnancy.

MATERIALS AND METHODS

Tissue and blood samples

Samples of myometrium, decidua and placenta were obtained from thirteen healthy women undergoing elective Caesarian sections at 38–42 weeks of pregnancy and placed immediately into ice-cold Krebs-Ringer solution as described previously (Batra & Bengtsson, 1976). Similar samples were obtained from nine patients undergoing abortion by hysterectomy in the 16th–22nd week of pregnancy. After rinsing thoroughly in Krebs-Ringer medium, samples of myometrium and placenta weighing 200–400 mg and the whole amount of decidua were blotted dry, weighed and frozen at −20°C, until used for analysis. We have previously shown that no significant loss of either steroid occurs from the tissues when they are stored at 4°C for 1 h (Batra, 1976).

Samples of venous blood (about 10 ml) were collected into heparinized glass tubes, centrifuged and the plasma stored frozen at −20°C until analysed.

Tissue digestion and extraction of steroids

The frozen tissues were thawed and then digested in 0·5 ml mixture containing 5% sodium dodecyl sulphate (SDS) and 0·5 N NaOH as described previously (Batra & Bengtsson, 1976). The digested material was extracted three times with 3 volumes of ethyl acetate; the combined extracts were evaporated to dryness under air at 40°C. The dried extract, however, contained a significant amount of SDS which was removed before radioimmunoassay (RIA) by Sephadex LH-20 chromatography, and 6 ml eluate were collected (Batra & Bengtsson, 1976; Batra, 1976).

Radioimmunoassay (RIA)

After evaporation of the solvent, the residue from the eluate was dissolved in 1 ml ethyl acetate, and a suitable amount, depending on the predicted concentration of oestradiol and progesterone, was taken for the respective RIA. The procedure for RIA of oestradiol was that described by Lindberg *et al.* (1974). We have previously evaluated the reliability of this assay for determining oestradiol in tissue extracts (Batra, 1976). The recovery of authentic unlabelled oestradiol added to digested samples and calculated at the end of the completed procedure was 88·5% (Batra, 1976). The coefficient of variation in replicate analysis of oestradiol was about 10%.

The method for determining progesterone was essentially that described by Youssefnejadian *et al.* (1972). The antiserum (FO 22.5.73), which was a gift from Dr Kjell Martinsson (Royal College of Veterinary Medicine, Stockholm) and found to be highly specific for progesterone, was used in a dilution of 1/1500 (vol/vol). Other details have

been described previously (Batra & Bengtsson, 1976). There was an almost complete recovery of not only radiolabelled progesterone, but also of unlabelled progesterone (96·5%) added to digested tissue and determined by RIA after extraction (Batra & Bengtsson, 1976).

Protein concentration in the digested tissue was determined by the method of Lowry *et al.* (1951) by using bovine serum albumin, dissolved in digestion mixture, as standard.

Chemicals

Sodium dodecyl sulphate (SDS) was purchased from Sigma Chemical Co. Oestradiol and progesterone were purchased from Ikapharm, Israel. Radioactive 2,4,6,7-H^3 oestradiol (102 Ci/mmol) and 1,2,6,7-H^3 progesterone (105 Ci/mmol) were obtained from New England Nuclear Corporation, and Sephadex LH-20 from Pharmacia Fine Chemicals, Sweden.

Statistics

The mean values from different groups were compared according to Student's *t* test.

RESULTS

Table 1 shows the mean concentrations of oestradiol and progesterone in plasma (per ml), decidua, myometrium and placenta (per g weight). Plasma oestradiol increased 4 fold from mid-term to term, whereas the placenta oestradiol just about doubled. There was also a significant increase in the oestradiol concentration in both myometrium and decidua. At mid-term there was no significant difference between oestradiol concentration in decidua and myometrium and concentrations in both these tissues were much smaller than that in the plasma.

In the case of progesterone there were some important differences in these interrelationships. Although, like oestradiol, the plasma concentration of progesterone rose towards term, the placental progesterone did not increase (but see also Table 4). The concentration

Table 1. Oestradiol and progesterone concentration in plasma (ng/ml), decidua, myometrium and placenta (ng/g wet weight) at mid-term and term

	Mid-term ($n=8$)		Term ($n=13$)	
Sample	Oestradiol	Progesterone	Oestradiol	Progesterone
Plasma	5·13 ± 1·33	38·84 ± 7·78	19·77 ± 0·87**	172·91 ± 15·73**
Decidua	0·67 ± 0·07	90·84 ± 17·28	1·47 ± 0·15**	170·57 ± 17·97*
Myometrium	0·70 ± 0·11	57·26 ± 6·78	2·31 ± 0·25**	76·91 ± 6·30
Placenta	6·43 ± 1·10	2061·77 ± 124·98	13·85 ± 1·51**	2443·51 ± 247·91

Values are means ± SE, and the significance of difference between mid-term and term is indicated by * $P<0·05$, ** $P<0·005$.

of progesterone in the decidua and myometrium at mid-term were 2–3 times higher than the concentration in plasma. At term, however, concentrations of progesterone in neither of these tissues exceeded plasma concentrations. Whereas the increase in decidual progesterone concentrations from mid-term to term was significant, it was not for myometrial progesterone.

The quantitative relationships between plasma and the tissue concentrations of either steroid are shown in Table 2. These data show that the capacity of decidua and myometrium for oestradiol accumulation is very low. Due to a large increase in plasma oestradiol concentration and relatively little increase in the tissue concentrations as the pregnancy advanced from mid-term to term, the tissue/plasma ratio in the case of both decidua and myometrium is further reduced. The concentration of progesterone in decidua and myometrium at mid-term was 3·5 and 1·9 times respectively higher than that in the plasma (Table 2). However there was a significant reduction in these ratios when the pregnancy advanced from mid-term to term.

Table 3 shows the relative concentration of progesterone and oestradiol in plasma and in the tissues at both mid-term and term. Plasma concentration of progesterone was about 8 times higher than oestradiol at both mid-term and term. Although the change in the

Table 2. Quantitative relationships between tissue (ng/g) and plasma (ng/ml) concentrations of oestradiol and progesterone at mid-term and term

	Decidua/plasma		Myometrium/plasma	
Pregnancy length	Oestradiol	Progesterone	Oestradiol	Progesterone
Mid-term ($n=9$)	0·19 ± 0·04	3·48 ± 1·43	0·17 ± 0·03	1·88 ± 0·30
Term ($n=13$)	0·08 ± 0·01**	1·03 ± 0·12*	0·12 ± 0·01	0·47 ± 0·04**

Values are means ± SE, and the significance of difference between mid-term and term is indicated by * $P<0·05$, ** $P<0·005$.

Table 3. Relative concentrations of progesterone and oestradiol (progesterone/oestradiol) in different tissues at mid-term and term

Pregnancy length	Plasma	Decidua	Myometrium	Placenta
Mid-term ($n=9$)	8·7 ± 1·3	112·2 ± 20·2	61·4 ± 11·4	370·0 ± 43·4
Term ($n=13$)	8·7 ± 0·6	124·3 ± 15·2	36·1 ± 3·4*	187·7 ± 20·3**

Values are means ± SE, and the significance of difference between mid-term and term is indicated by * $P<0·05$, ** $P<0·005$.

relative concentration of steroids was insignificant in decidua it was significant in myometrium. The progesterone/oestradiol ratio in placenta was almost halved from mid-term to term and this reduction was statistically significant.

When steroid concentrations in the tissues were calculated on the basis of protein content it was found that although the general pattern was similar to that shown in Table 1 (per gram wet weight), the quantitative relationships were somewhat changed. This was due to the difference in protein concentrations per unit weight in different tissues (Fig. 1). Firstly, the protein concentration in the myometrium at mid-term was significantly higher than that in the decidua or placenta. Secondly, whereas the protein concentrations in both placenta and decidua increased significantly from mid-term to term, that in the myometrium remained virtually unchanged. This resulted in the fact that at term the difference

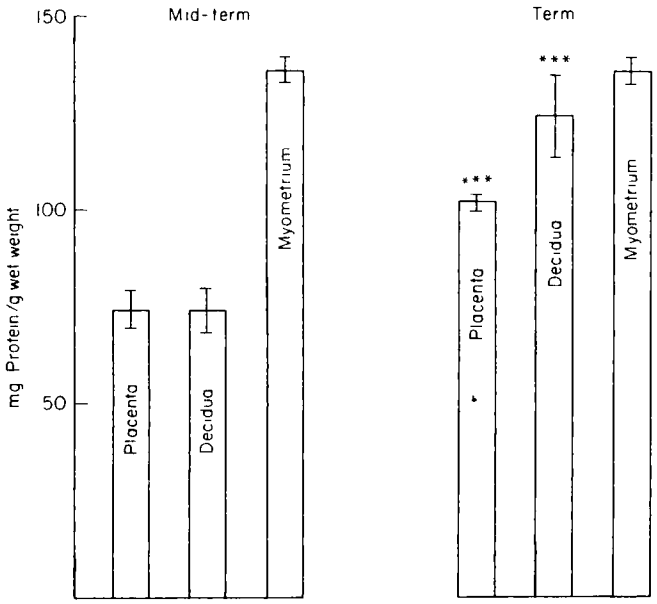

Fig. 1. Protein content per g wet weight (mean ± SE) in different tissues at mid-term ($n = 9$) and term ($n = 13$), *** $P < 0.005$.

between protein concentrations in myometrium and decidua was no longer significant but the difference in myometrium and placenta was still significant.

When oestradiol concentrations were expressed in terms of protein (Table 4), the myometrial oestradiol concentration decreased in relation to placental and decidual concentrations. This was most marked at mid-term. Now, oestradiol concentration in the myometrium was lower than that in the decidua (compare with Table 1). Furthermore, the difference between placental concentrations at mid-term and term became less significant and that between decidual concentrations became insignificant (compare with Table 1).

In the case of progesterone (Table 4) a similar change occurred but no important difference in the quantitative relationships between different tissues was revealed (compare with Table 1).

Table 4. Oestradiol and progesterone concentration (ng/mg protein) in decidua, myometrium and placenta at mid-term and term

	Mid-term ($n=8$)		Term ($n=13$)	
Sample	Oestradiol	Progesterone	Oestradiol	Progesterone
Decidua	0·009 ± 0·001	1·18 ± 0·14	0·013 ± 0·001	1·57 ± 0·23
Myometrium	0·005 ± 0·001	0·43 ± 0·006	0·017 ± 0·002**	0·56 ± 0·04
Placenta	0·089 ± 0·015	28·72 ± 2·58	0·138 ± 0·017*	24·21 ± 2·61

Values are means ± SE, and the significance of difference between mid-term term and term is indicated by * $P<0·05$, ** $P<0·005$.

DISCUSSION

There has been a general trend while studying the effects of oestrogens and progesterone on various female reproductive tissues to relate the intensity of the effect to the concentration of hormones in the blood. It is, however, more reasonable to think that the biological activity will perhaps be governed by concentrations in the tissue. A recent study from this laboratory showed that the concentration of oestradiol and progesterone in the myometrium of pregnancy did not reflect the concentration found in the plasma (Batra & Bengtsson, 1978). The results of the present study show that the decidual concentration of oestradiol, as that of the myometrium, during pregnancy is very low as compared with that of plasma (Table 1). In fact at full term, decidual oestradiol concentration was lower than the myometrial concentration, indicating extremely low capacity of decidua for oestradiol accumulation. This might be the result of progesterone influence on oestradiol binding in the tissue, as previously suggested for the myometrium (Batra & Bengtsson, 1978). The data on progesterone concentrations in these tissues support this suggestion. Whereas the progesterone concentration in the decidua from mid-term to term nearly doubled, the increase in myometrium was not significant (Table 1). There are no other data published on the concentrations of these steroids in the decidua. The data on the concentrations in the myometrium are in general agreement with other available data (Runnebaum & Zander, 1971; Batra & Bengtsson, 1978; Ferré *et al.*, 1978).

The relatively low decidual and myometrial oestradiol concentrations in pregnancy and the indication of only minor changes towards term might be taken as an argument against a direct or dominant role of this steroid in the onset of labour. On the other hand the high concentration of progesterone in the decidua that increased significantly at term might be considered to support the argument for its role in the control of parturition. However, progesterone is assumed to stabilize the lysosomes of the decidual and fetal membranes, while the proposed mechanism for the increased production of prostaglandins is *via* the labilization of lysosomes (Gustavii, 1975; Schwarz *et al.*, 1975). Milewich *et al.* (1977) have recently proposed that altered metabolism of progesterone and its binding

to a specific protein of progesterone in fetal membranes (Schwarz *et al.*, 1976) reduced the local concentration of progesterone that is available to act as a stabilizer on fetal membrane lysosomes. It would be interesting to know the endogenous levels of oestradiol and progesterone in fetal membranes, how they alter with advancing pregnancy and what is their relation to the concentrations in the decidua and myometrium. In this connection, the results showing that progesterone/oestradiol ratio in the myometrium (Table 3) decreased at term may be of importance. We have recently observed (unpublished data) that this ratio is higher in the myometrium of women at term than of those in labour.

The concentration of progesterone in the placenta was very high already at mid-term and did not show further increase at term (Table 1). This is in agreement with recent data of Runnebaum *et al.* (1975). The placental oestradiol concentration was unexpectedly low at mid-term compared to progesterone concentration (Table 1). However, in contrast to progesterone it increased significantly towards term (Table 1). This increase in placental oestradiol concentration might reflect fetal contribution. This seems logical in view of the fact that until around mid-term adrenals of the fetus are not fully developed and therefore the fetal supply of oestradiol precursors is limited. This is also compatible with the existing evidence that the production of progesterone by the placenta is more or less independent of the fetus, whereas oestradiol production during pregnancy is to a great extent dependent on the supply of precursors from the fetus.

Although the general relationship between the steroid concentrations in various tissues were well maintained when expressed in terms of their protein content (Table 4), it is of interest that the protein concentrations per unit weight can greatly vary between different tissues (Fig. 1). It was, however, not surprising to find that the myometrium, which is a muscular tissue, had higher protein content than tissues devoid of muscle such as placenta and decidua. The finding that the protein concentration in placenta and decidua, in contrast to myometrium, increased from mid-term to term is somewhat puzzling. One explanation for this might be that hypertrophy exceeds hyperplasia under the continuous influence of oestradiol and progesterone. Another possibility might be that there is a loss of fluid in these tissues after mid-term. This later explanation is consistent with the general observation that placenta in early pregnancy is less solid than at term.

The present study provides further evidence that the plasma concentration of oestradiol and progesterone is not directly related to their tissue concentration. We have also shown that the capacity of both the myometrium and the decidua for oestradiol accumulation is much lower than for progesterone.

ACKNOWLEDGMENTS

We are grateful to Mrs Kristina Källstrand, Mrs Bodil Nilsson and Mrs Iréne Larsson for their skillful technical assistance. This work was supported by the Ford Foundation and the Swedish Medical Research Council (project No. 4781).

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