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Neuroactive steroids, their precursors and polar conjugates during parturition and postpartum in maternal and umbilical blood: 3.3β-hydroxy-5-ene steroids

M. Hill [a,*], A. Pařízek [b], J. Klak [a], R. Hampl [a], J. Šulcová [a], H. Havlíková [a], O. Lapčík [a], M. Bičíková [a], T. Fait [c], R. Kancheva [a], D. Cibula [b], V. Pouzar [d], M. Meloun [e], L. Stárka [a]

[a] *Institute of Endocrinology, Národní třída 8, Prague, CZ 116 94, Czech Republic*
[b] *Clinics of Gynecology and Obstetrics, First Medical School, Charles University, Prague, Czech Republic*
[c] *General Hospital, First Medical School, Charles University, Apolinářská 18, CZ 128 51, Prague, Czech Republic*
[d] *Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic*
[e] *Institute of Analytical Chemistry, Faculty of Chemical Technology, Pardubice University, Pardubice, Czech Republic*

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Abstract

Five 3β-hydroxy-5-ene steroids involved in the metabolic route from pregnenolone sulfate to dehydroepiandrosterone and its sulfate, of which three are known allosteric modulators of neurotransmitter receptors, were monitored in the serum of 20 women around parturition. In addition, their levels in maternal and umbilical serum were compared at delivery. On the basis of these data, a scheme of steroid biosynthesis in maternal organism during the critical stages around parturition is proposed.

In maternal serum, all the steroids except dehydroepiandrosterone sulfate decreased during labor and even first day after delivery, although their changes were less distinct the more distant from pregnenolone sulfate (PregS) in the metabolic pathway. Calculation of product/immediate precursor ratios in maternal serum over all stages around parturition enabled identification of the respective changes in the activities of the relevant enzymes. The ratio of 17-hydroxypregnenolone/pregnenolone did not change significantly, while that of dehydroepiandrosterone/17-hydroxypregnenolone grew, indicating increased C17,20 side chain cleavage on the account of C17-hydroxylation both catalyzed by C17-hydroxylase-C17,20-lyase. As was shown by factor analysis, the changes in the maternal steroids were associated with a single common factor, which strongly correlated with all the steroids except dehydroepiandrosterone sulfate. The lack of change in the pregnenolone sulfate/pregnenolone ratio and a marked increase of the ratio dehydroepiandrosterone sulfate to unconjugated dehydroepiandrosterone indicate a different means of formation of both steroid sulfates. On the basis of these data, a scheme of steroid biosynthesis in maternal organism during the critical stages around parturition is proposed.

Keywords: Parturition; Time profiles; 3β-Hydroxy-5-ene steroids; Steroid sulfates

1. Introduction

Considering the physiological events in maternal organism shortly before and at term, characterized by cooperation of various hormonal systems from both maternal and fetal compartment, among other things by an enormous release of stress hormones [1], of importance may be also steroids, which, in different way, influence the neuronal function in both brain and periphery. Generally, endogenous factors with anxiolytic–analgetic properties acting in brain are needed to attenuate the perception of the pain, while others are necessary for activation of neural transport in the periphery.

The fetal adrenal cortex produces predominantly 3β-hydroxy-5-ene steroids and their sulfates, which are used by the placenta for estrogen synthesis [2]. Sulfates of 3β-hydroxy-5-ene steroids belong to effective modulators of neurotransmitter receptors influencing the permeability of ion channels [3–9]. Pregnenolone sulfate (PregS) is a well-known activator of the membrane *N*-methyl-D-aspartate (NMDA) receptors regulating the permeability of calcium channels [6,7,10]. Moreover, PregS and dehydroepiandrosterone sulfate (DHEAS) are allosteric inhibitors of the $GABA_A$ receptors responsible for the attenuation of neuronal activity by modulating chloride influx [5,11–15]

* Corresponding author. Tel.: +420-224905267; fax: +420-224905325.
E-mail address: mhill@endo.cz (M. Hill).

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while the unconjugated DHEA acts in an opposite way. A number of in vitro experiments have been reported, as well as studies on animals, considering the mechanism of the action and neuromodulating effects of 3β-hydroxy-5-ene steroids. The in vitro investigations, however, are frequently biased by artificial experimental conditions. The steroid metabolism in experimental animals usually differs significantly from that in humans, particularly as concerns the steroid sulfatase/sulfotransferase system and the action of steroid C17-hydroxylase/C17,20 lyase. Several authors have investigated the changes of selected 3β-hydroxy-5-ene steroids in humans either during pregnancy or around term [16–28]. However, no studies have involved a complete 3β-hydroxy-5-ene pathway.

The authors' aim was to evaluate simultaneous time profiles of the relevant 3β-hydroxy-5-ene steroids—namely pregnenolone (3β-hydroxy-5-pregnen-20-one, Preg), its sulfate (PregS), 17-hydroxypregnenolone (3β,17α-dihydroxy-5-pregnen-20-one, Preg17), dehydroepiandrosterone (3β-hydroxy-5-androsten-17-one, DHEA) and its sulfate (DHEAS)—in women around parturition. On the basis of the changes in product/precursor ratios, an attempt was made to trace changes in steroid enzyme activities around parturition and postpartum which may be associated with the timing and course of labor. The differences in steroid levels between maternal and umbilical blood, as well as their correlations at delivery, were calculated with respect to a possible transport between both compartments. The following questions were addressed.

- Do the levels of 3β-hydroxy-5-ene steroids and the proportions of conjugated (sulfated) and unconjugated steroids in the maternal serum change during the period around parturition and at delivery, and if so, how?
- Do the levels and proportions of conjugated (sulfated) and unconjugated steroids differ between maternal and umbilical serum at delivery, and if so, how?
- Are there any correlations between the individual steroids as measured either in maternal or umbilical serum?

2. Experimental

2.1. Subjects

The patient group consisted of 20 women at delivery, of whom 13 were treated with subarachnoidal and 7 with epidural analgesia, respectively. Both types of analgesia have been described in detail elsewhere [29].

Informed written consent was obtained from all of the subjects both for the collection and utilization of the samples.

2.2. Sample collection

Samples of maternal blood were collected from the cubital vein in five stages during parturition and in the postpartum period. The first stage named "Cervical dilatation 3 cm" was characterized by a diameter of the os uteri of 3–4 cm. The border that remained of the os uteri after 30 min from the "Cervical dilatation 3 cm" stage, when the cervical dilatation reached a diameter of 10–11 cm, identified the second stage, named "Cervical dilatation 11 cm". The third, fourth and fifth stages, named "1 h after", "1 Day after" and "5 Days after", corresponded to samples collected 1 h, 1 day and 5 days after delivery, respectively. In addition, the samples of mixed arterial and venous umbilical blood were withdrawn at labor. Each sample was collected to a cooled plastic tube containing 100 ml of 5% EDTA and 50 pl of aprotinin (Antilysin from Spofa, Prague, Czech Republic). The serum was obtained by centrifugation at 2000 × g and 0 °C for 5 min. The samples of serum were stored at −20 °C until analyzed.

2.3. Steroids and chemicals

Non-radioactive steroids and their conjugates were purchased from Steraloids (Wilton, NH, USA). The solvents for extraction and HPLC were of analytical grade, from MERCK (Darmstadt, Germany).

2.4. Instruments

The Gilson (France) HPLC system consisted of a 305 pump with 805 manometric, 306 slave pump, 811C dynamic mixer, 234 autoinjector and FC 203B fraction collector. The LCD 2082 UV detector and LCO 100 column oven were from ECOM (Czech Republic). The ET 250/4 NUCLEOSIL® 100-5 C18 reverse phase column was from Macherey-Nägel (FRG). A DataApex (Czech Republic) CSW APEX system was used for the collecting and treatment of chromatographic data. The liquid scintillation spectrometer was supplied by BECKMANN INSTRUMENTS, (Fullerton, CA, USA).

2.5. Analytical methods

The method for determination of pregnenolone sulfate has been described elsewhere [30]. Pregnenolone and 17-hydroxypregnenolone were determined by sensitive RIA methods following HPLC separation [31]. DHEA and DHEAS were measured using RIA kits from Immunotech (Marseille, France).

2.6. Statistical evaluation of the data

For the evaluation of changes in the time profiles, two-way ANOVA was used with stage and subject as factors A and B, respectively. Tukey interaction between the factors was not included in the model. For hypothesis testing, it was strictly assumed that the model error is normally and independently distributed, and a homoscedasticity or constant variance throughout the level treatments is supposed.

Because of the non-Gaussian distribution in the concentrations of all of the measured steroids, the original data were subjected to a power transformation to attain the minimum skewness of the normalized residuals [32,33]. To avoid the influence of outliers normalized residues with absolute values >2 were excluded from the calculations. The group means calculated from the transformed data with their lower and upper limits of confidence intervals were re-transformed to the original scale, and the values thus obtained were used for a graphical demonstration of the time profiles. The re-transformed mean values were close to the medians and their confidence intervals were more or less asymmetrical, reflecting the skewness of the original data. Individual differences between the stages were evaluated by the use of least significant differences multiple comparisons.

Spearman's robust pair and partial correlations (with the adjustment to constant levels of all steroids other than the pair evaluated) were used to evaluate relationships between the steroids. Spearman's correlation matrix was used as a basis for a subsequent factor analysis. The differences in the steroid levels between maternal and umbilical serum were evaluated with the use of Student's paired *t*-test and Wilcoxon's robust paired test.

With the exception of the correlation analysis, which was carried out using NCSS 2000 statistical software (NCSS, Kaysville, UT, USA), all remaining computations were performed using Statgraphics Plus 3.3 (Manugistics Rockville, MA, USA).

3. Results

3.1. Time profiles of the steroids

The time profiles of the steroids are shown in Fig. 1. All the steroids exhibited significant changes during parturition and postpartum. The changes were most marked in PregS, and they were less distinct the more distant from that steroid. In contrast to PregS, the profile of which strongly resembled those in unconjugated Preg and Preg17, the DHEAS profile was different. The between-stage differences in DHEAS were insignificant except for the stage "1 Day after delivery"; in which the levels of DHEAS were significantly lower than the levels of the preceding and succeeding stages; "5 Days after delivery', they reached the same value as at the beginning of labor. The DHEA profile resembled those of pregnenolone and Preg17 and differed significantly from that of DHEAS, excepting levels at the fifth day, which were also significantly higher than in the first day, although still not reaching the value before delivery.

3.2. Time profiles of the steroid product/precursor ratios

The changes in steroid product/precursor ratios are shown in Fig. 2. As demonstrated by Fig. 2, the Preg17/Preg ratio did not exhibit any significant changes around parturition. A significantly increasing trend was found in the DHEA/Preg17 ratio, however, the value of which increased more than three times on the first day after delivery in comparison with the "Cervical dilatation 3 cm" stage.

3.3. Time profiles of the steroid sulfate/unconjugated steroid ratios

As demonstrated by Fig. 3, the PregS/Preg ratio did not exhibit any significant changes while the DHEAS/DHEA ratio increased by about three times within first hour and first day after delivery.

3.4. Differences between maternal and umbilical serum in the individual steroids at delivery

The levels of the five studied steroids in maternal and fetal serum at delivery and the respective statistical characteristics are shown in Fig. 4. Significantly higher levels of PregS and Preg as well as insignificantly higher levels of Preg17 were found in umbilical serum, while no difference was observed in DHEA and DHEAS. The variance of DHEA in maternal serum was greater than that in umbilical serum.

3.5. Differences in steroid product/precursor ratios for maternal and umbilical serum at delivery

The differences between the proportions of the products and their direct precursors in maternal and umbilical serum at delivery are shown in Fig. 5. Both the Preg17/Preg and DHEA/Preg17 ratios were lower in the umbilical serum.

3.6. Differences between the proportions of conjugated and unconjugated steroids in maternal and umbilical serum at delivery

No differences between the proportions of the conjugated and unconjugated steroids in maternal and in umbilical serum at delivery were found for either Preg or DHEA.

3.7. Mutual correlations of steroid levels in maternal plasma

Table 1 shows the Spearman's correlation matrix for each pair of the corresponding values of maternal steroid levels, including all the stages around parturition. All the steroids except DHEAS were more or less correlated. Maximum correlations occurred between Preg and Preg17 and between PregS and Preg, respectively, while DHEAS correlated only with DHEA.

3.8. Factor analysis of the correlations in maternal serum

On the base of the correlation analysis, factor analysis was performed in order to evaluate the statistical

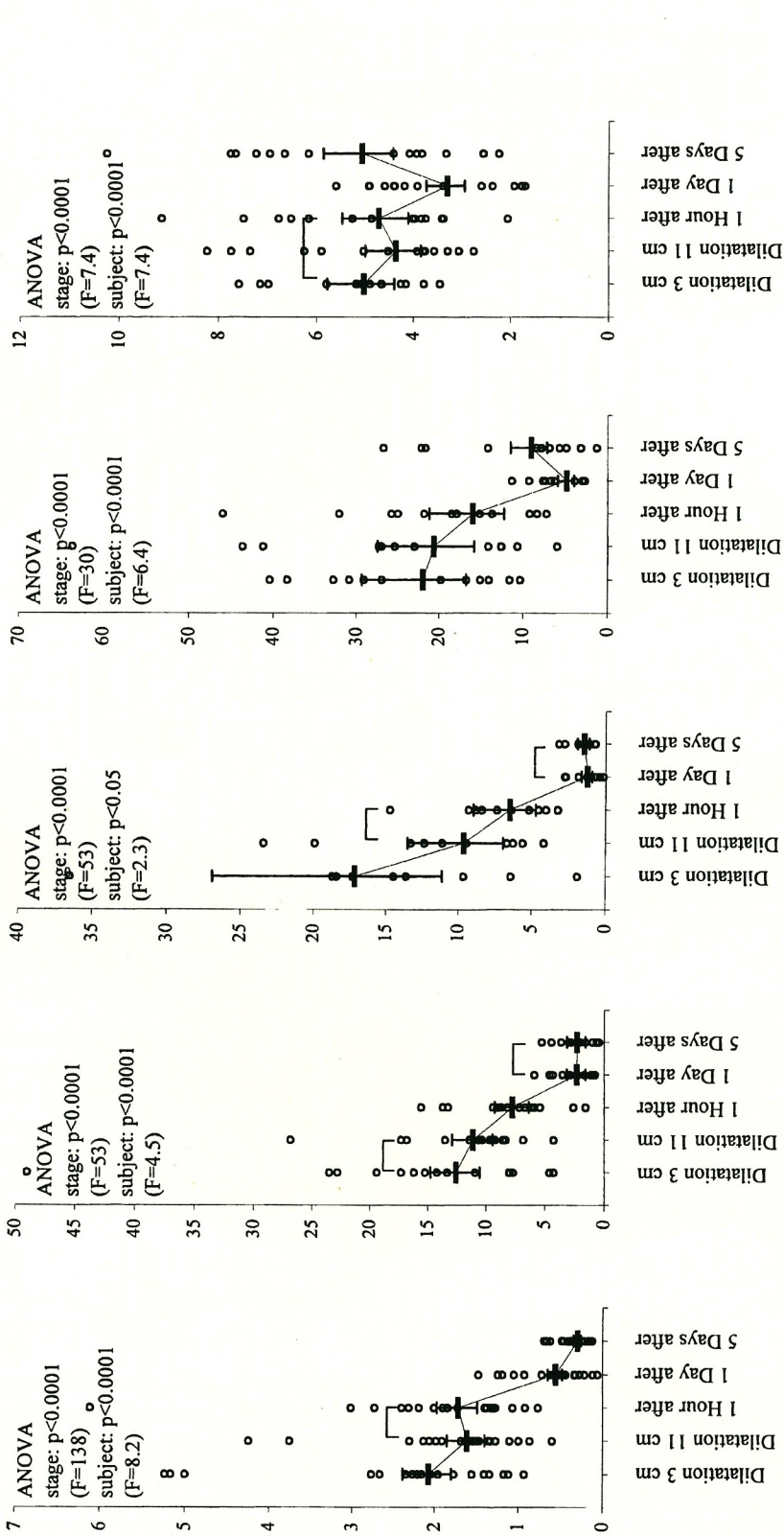

Fig. 1. Time profiles of five 3β-hydroxy-5-ene steroids in maternal serum around parturition. The short horizontal lines with error bars represent group mean values with 95% confidence intervals calculated using least significant difference multiple comparisons. Clamps denote groups with insignificant differences between mean values, P is the level of statistical significance and F is the explained/random variance ratio (for statistical treatment see Section 2.6).

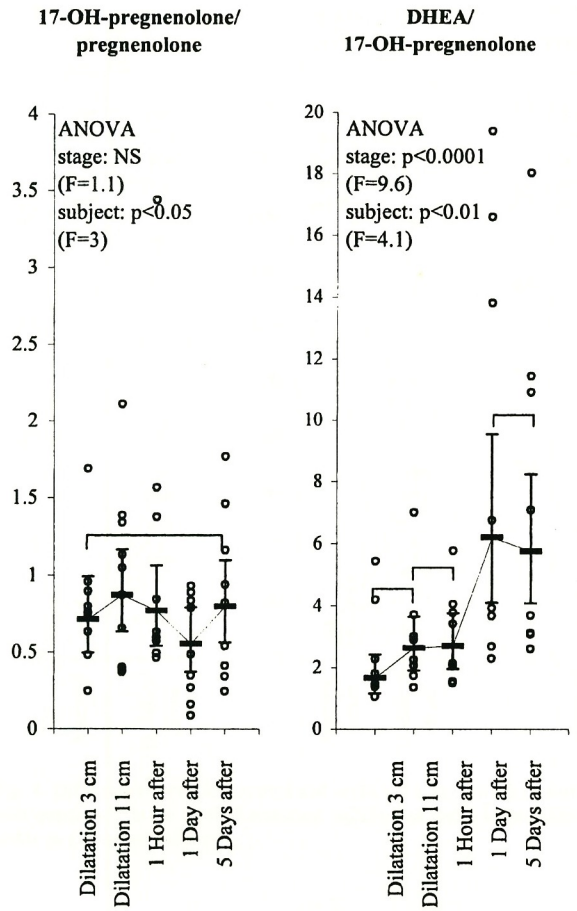

Fig. 2. Time profiles for the product/precursor ratios of four unconjugated 3β-hydroxy-5-ene steroids in maternal serum around parturition. The drawings and symbols are the same as for Fig. 1.

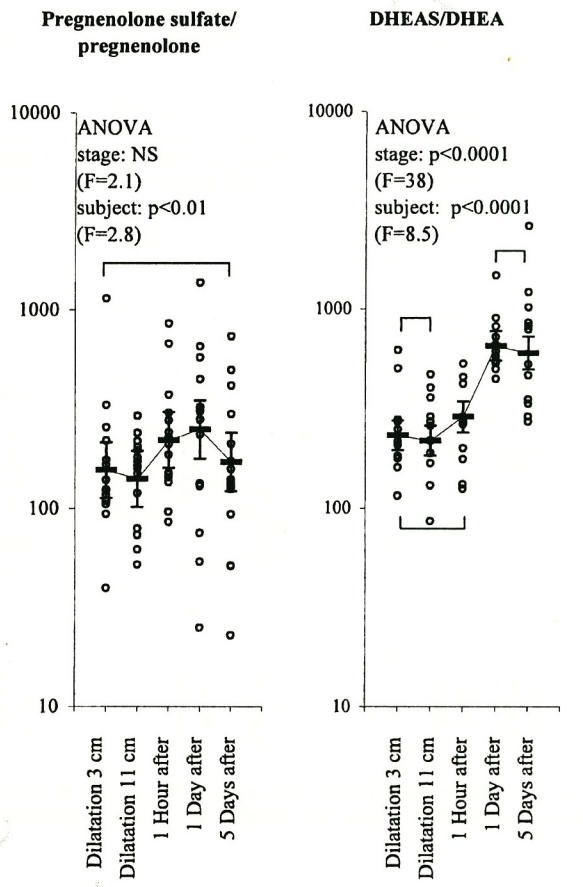

Fig. 3. Time profiles for the sulfated/unconjugated 3β-hydroxy-5-ene steroid ratios in maternal serum around parturition. The drawings and symbols are the same as for Fig. 1.

weight of said correlations and to find the structure of the relationships between the variables. Taking into consideration 39 complete records from the total of 90, a single factor explaining 88% of the total variability was found, which was responsible for the changes. This factor strongly correlated with all the steroids except DHEAS. The relevant eigenvalues and factor loadings are given in Table 2.

3.9. Correlations between steroid levels in maternal and umbilical serum at delivery

Table 3 shows Spearman's correlations of the steroids at delivery separately for maternal (section A) and umbilical serum (section B). Section C gives the correlations for the respective steroids in maternal and umbilical serum. In comparison with the time profiles around parturition, the correlations in maternal serum were markedly weaker. The only significant correlation was found between DHEAS and DHEA.

A slightly different situation than that in maternal serum was found in umbilical blood (Table 3, section B). A borderline positive correlation was found between PregS and Preg and a significant negative correlation was found between Preg and DHEAS.

No correlation was found between maternal and umbilical serum levels of the steroids except for a negative correlation between DHEAS in umbilical and DHEA in maternal serum (Table 3, section C).

4. Discussion

Two steroid sulfates involved in the metabolic pathway leading to DHEA, PregS and DHEAS are known allosteric modulators of some neuronal receptors (GABA, NMDA) and enhancers of neuronal activity, while DHEA itself acts in an opposite manner. As neuroactive steroids as well as precursors of major estrogens, they are important to the physiological course of pregnancy and may influence events around parturition.

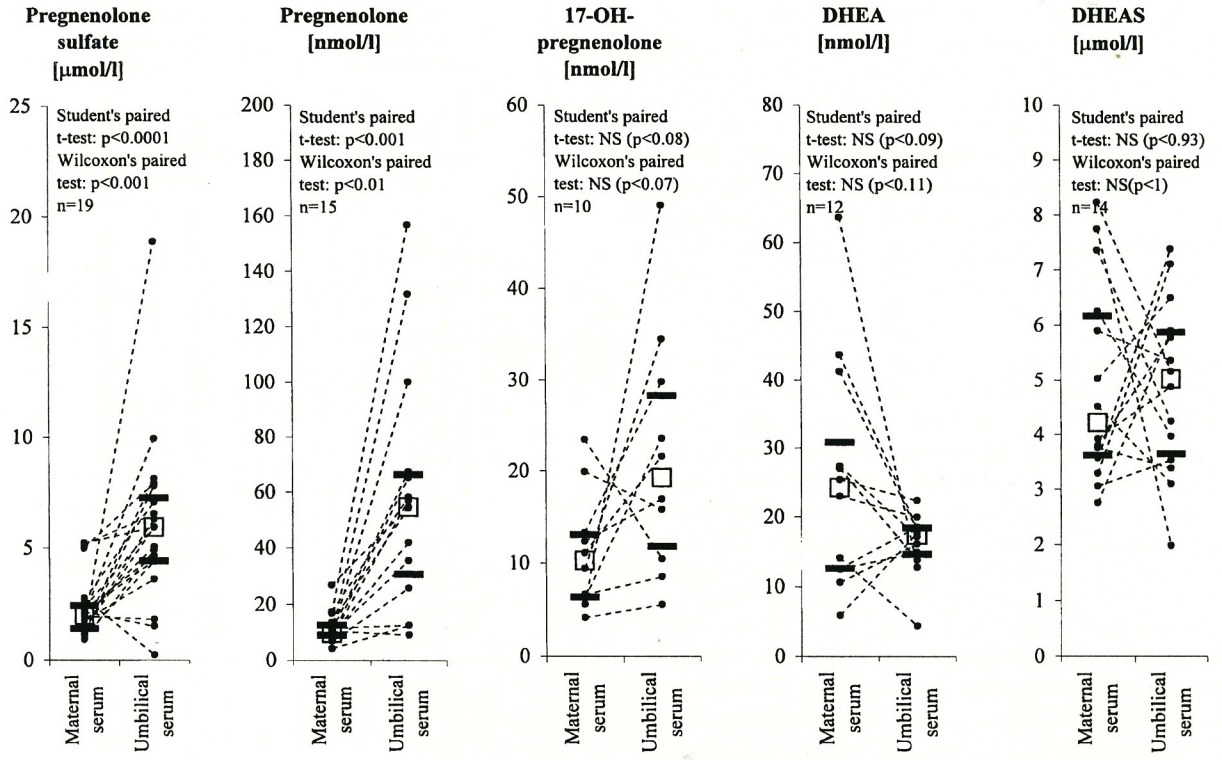

Fig. 4. Differences between maternal and umbilical serum in 3β-hydroxy-5-ene steroids at delivery. The empty squares with error bars represent medians with quartiles, *P* is the level of statistical significance and *n* is the number of subjects. Dashed lines join the corresponding maternal and umbilical steroid levels in individual subjects.

Table 1
Spearman's correlations between pregnenolone sulfate (PregS), pregnenolone (Preg), 17-hydroxypregnenolone (Preg17), dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) in maternal serum and in all stages of parturition and postpartum

PregS	0.4674	0.2523	−0.1530	0.1329
	0.0000	0.0607	0.2094	0.2369
	90	59	72	84
0.8092	Preg	0.5266	0.0783	−0.1633
0.0000		0.0000	0.5418	0.1615
90		59	66	78
0.7636	0.8638	Preg17	0.5029	−0.1744
0.0000	0.0000		0.0005	0.1987
59	59		47	59
0.4889	0.5871	0.7095	DHEA	0.5857
0.0000	0.0000	0.0000		0.0000
72	66	47		72
0.0970	0.0528	0.1396	0.5039	DHEAS
0.3801	0.6464	0.2918	0.0000	
84	78	59	72	

The values above and below the diagonal represents partial correlations between the pair of steroids with adjustment to constant level of the remaining variables and simple pair correlations, respectively. The values in the first second and third rows represent Spearman's correlation coefficient, its level of statistical significance and number of subjects, respectively.

Table 2
Factor analysis of Spearman's correlations between pregnenolone sulfate (PregS), pregnenolone (Preg), 17-hydroxypregnenolone (Preg17), dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) in maternal serum and in all stages of parturition and postpartum

Factor number	Eigenvalue	Percent of variance	Cumulative percentage
1	3.0	88.0	88.0
2	0.4	11.4	99.4
3	0.0	0.6	100.0
4	0.0	0.0	100.0
5	0.0	0.0	100.0

Steroids	Communality		Factor loadings
	Initial variable	Estimated variable	
PregS	0.639	0.652	0.807
Preg	0.787	0.776	0.881
Preg17	0.759	0.809	0.899
DHEA	0.708	0.726	0.852
DHEAS	0.297	0.057	0.239

Only complete records (39 from a total of 90) were included in the factor analysis. Factor loadings represent correlations between variables and the factor.

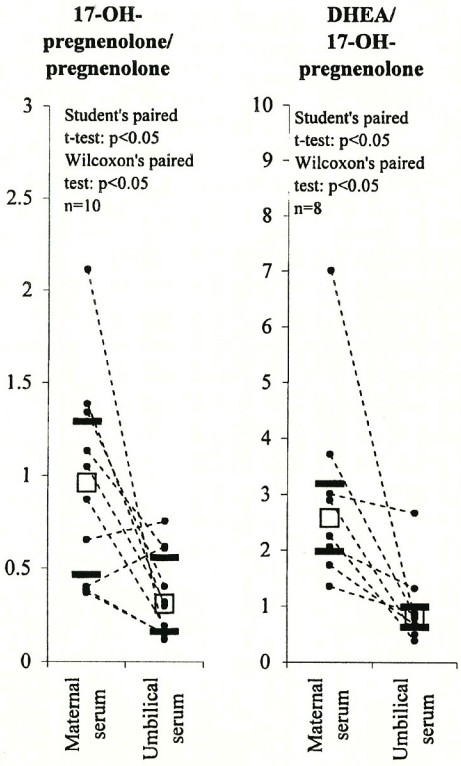

Fig. 5. Differences between maternal and umbilical serum in the product/precursor ratios of 3β-hydroxy-5-ene steroids at delivery. The drawings and symbols are the same as for Fig. 4.

In this project, time changes in serum levels of five relevant 3β-hydroxy-5-ene steroids (PregS, Preg, Preg17, DHEA and DHEAS) were systematically followed in 20 women during the short stretch of time preceding delivery and in the post-partum period. In addition, the levels of these steroids were determined in umbilical serum at delivery and compared with those in the maternal serum.

The levels of all of these steroids—with the exception of DHEAS in maternal serum—decreased during labor and first day after delivery, although the changes were less pronounced the more remote they were from PregS in the metabolic pathway (see Fig. 1).

The activities of the responsible enzymes were followed by calculation of the product/precursor ratios and the tightness of their mutual correlations. The finding that the Preg17/Preg ratio around parturition did not significantly change while the DHEA/Preg17 ratio increased significantly (Fig. 2) agreed with the observation of other authors studying C17-hydroxylase/17,20-lyase (P450C17) activities that high levels of the substrate versus enzyme concentration leads to greater hydroxylation activity whereas the reverse increases the C17,20-side chain cleavage activity [34]. There were highly significant correlations between the steroid pairs in the sequence PregS to DHEA, these being the tightest for each product-precursor pair (Table 1), while there was no correlation between DHEAS and other steroids except DHEA. When the partial correlations were computed with adjustment to constant level of the all steroids except the pair evaluated, the significant correlations remained only between products and their immediate precursors. A

Table 3
Spearman's correlations between levels of pregnenolone sulfate (PregS), pregnenolone (Preg), 17-hydroxypregnenolone (Preg17), dehydroepiandrosterone (DHEAS) and dehydroepiandrosterone sulfate (DHEAS) in maternal and umbilical serum at delivery

Maternal serum (section A)					Umbilical serum (section B)					Maternal vs. umbilical serum (section C)	
PregS	0.3490	0.2613	−0.4093	0.0584	PregS	0.3720	0.0241	−0.0429	0.1878	0.0211	PregS
	0.2662	0.5715	0.2740	0.8646		0.2337	0.9591	0.9127	0.5803	0.9298	
	15	10	12	14		15	10	12	14	20	
0.1357	Preg	−0.0179	0.5972	−0.2701	0.5107	Preg	0.1861	0.6962	−0.8496	0.2393	Preg
0.6296		0.9697	0.1180	0.4505	0.0517		0.6895	0.0551	0.0019	0.3904	
15		10	11	13	15		10	11	13	15	
0.1758	0.1394	Preg17	0.1068	0.2435	0.2848	0.4303	Preg17	0.1622	0.0511	−0.0788	Preg17
0.6272	0.7009		0.8643	0.5988	0.4250	0.2145		0.7944	0.9134	0.8287	
10	10		8	10	10	10		8	10	10	
−0.2867	0.5000	0.2619	DHEA	0.5951	0.3357	0.4000	0.4286	DHEA	0.7460	−0.0140	DHEA
0.3663	0.1173	0.5309		0.0909	0.2861	0.2229	0.2894		0.0210	0.9656	
12	11	8		12	12	11	8		12	12	
−0.1560	0.1429	0.3576	0.6294	DHEAS	−0.1692	−0.6484	−0.0909	0.2797	DHEAS	−0.3670	DHEAS
0.5942	0.6415	0.3104	0.0283		0.5630	0.0165	0.8028	0.3786		0.1967	
14	13	10	12		14	13	10	12		14	

The values above the diagonals in the first and second sections represent partial correlations between two steroids with adjustment to constant levels of the remaining steroids, while the values below the diagonals and in the third section express correlations without such adjustments. The values in the first, second and third rows represent Spearman's correlation coefficient, its level of statistical significance and number of subjects, respectively.

borderline partial correlation between PregS and Preg17 still allow a possibility of adjacent metabolism of PregS to Preg17 via 17-hydroxypregnenolone sulfate. However, its significance is minor in comparison with the route via unconjugated pregnenolone.

The lack of change in the PregS/Preg ratio and at the same time the marked growth of the DHEAS/DHEA ratio points to a different method of formation for both steroid sulfates (Fig. 3) [35–37]: a direct conversion of cholesterol sulfate to pregnenolone sulfate proceeding concurrently with a conversion of unconjugated cholesterol to pregnenolone has been reported [33,35], while in the case of DHEAS it is known that adrenal DHEAS is formed by the sulfatation of DHEA by the action of sulfotransferase, present in a high concentration in the *zona reticularis*, as well as by a direct cleavage of the side chain of cholesterol sulfate on carbon 17 [38,39]. A different source of PregS and DHEAS is also demonstrated by the lack of correlation between them (Table 1). To reveal the interplay of the relationships between the profiles of the steroids a factor analysis over all stages of parturition was performed. The correlation analysis as well as the results of the factor analysis revealed that only one factor was responsible for the changes in the levels of steroids in maternal serum around parturition. As shown in Table 2, this factor strongly correlated with all the steroids investigated except DHEAS, whereas PregS changed like most of steroids studied, further confirming the suggested interpretation.

The situation at delivery was different from that before and after term. The steroids in the maternal serum at delivery were surprisingly weakly correlated (Table 3, section B). The only significant correlation was found between DHEA and DHEAS. This may be explained by sulfatation of DHEA as the major enzymatic reaction resulting in DHEAS. There was no mutual correlation among the steroids between maternal and umbilical serum (Table 3, section C). Generally, significantly higher levels of PregS, Preg and Preg17 were found in umbilical serum than in maternal serum. The respective differences in DHEA and its sulfate were insignificant (see Fig. 4). When the product/substrate ratios were plotted for the Preg17/Preg and DHEA/Preg17 pairs (Fig. 5), significantly higher values were found in maternal serum, indicating a higher conversion rate in the mothers. No such a pattern appeared for the sulfated/unconjugated steroid ra-

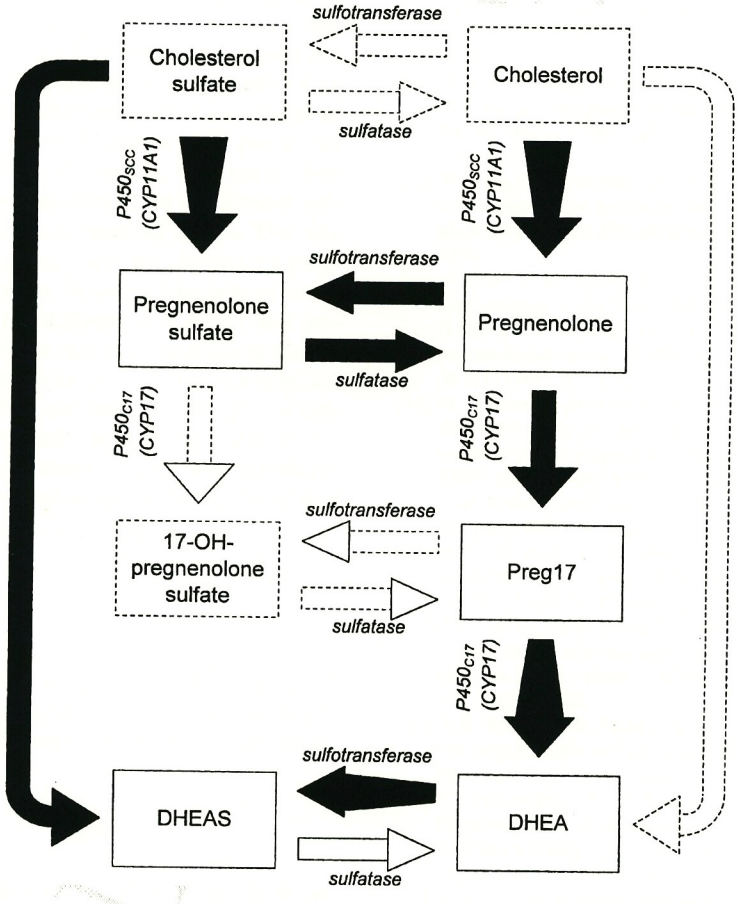

Fig. 6. Simplified scheme of maternal steroidogenesis derived from the steroid profiles in maternal serum. Full arrows represent the principal metabolic pathway. Dotted lines indicate supposed or missing data. The extension of arrow lines shows the direction of changes in steroid levels during parturition.

tios. The dashed lines on Figs. 4–6 connecting steroid levels and product/substrate ratios from individual subjects reflect the extent of the association between the respective values in maternal and umbilical serum. This was significantly higher in the first two steroids, PregS and Preg, and the similar trend was also indicated in Preg17. The situation was different in the C19 members of the biosynthetic route DHEA and DHEAS. However, no correlation between maternal and umbilical steroid levels was apparent (compare Table 3, section C) in any of the steroids. It should be emphasized that umbilical blood contains a mixture of steroids of placental as well as fetal origin, relatively autonomous in relation to the mother's circulation. The only correlations in umbilical serum were found for DHEA and its sulfate and, surprisingly, a negative correlation was found between DHEAS and pregnenolone (Table 2, section B). The latter negative correlation as well as the lack of any other correlation with the remaining steroids could be ascribed to competition between DHEAS and PregS for placental sulfatase.

In contrast to the results of the umbilical serum analysis, some conclusions can be drawn concerning changes of steroid concentrations and the relevant enzyme activities in maternal circulation around parturition: from the onset of labor until the first day after delivery the concentration of pregnenolone sulfate gradually sinks, this decline being paralleled by pregnenolone and 17α-hydroxypregnenolone. The rate of DHEA formation increases on account of its immediate precursor, 17α-hydroxypregnenolone. As far as DHEAS is concerned, its concentrations around parturition remain nearly constant; from the results, it is not evident whether this too is formed directly from cholesterol sulfate or predominantly by the sulfatation of DHEA. The only conclusion is that its formation does not depend on pregnenolone sulfate concentration. In spite of the lack of correlation between steroid concentrations in maternal and umbilical serum, their decline in maternal circulation during parturition and after delivery may be caused by the decrease and later absence of the fetal contribution. The physiological reason, however, is not clear. One may speculate that it is the response to the lack of stress. A suggested scheme of steroid biosynthesis in maternal organism during the critical stages around parturition is given in Fig. 6.

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