Is maternal progesterone actually independent of the fetal steroids?

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Short title:
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ABSTRACT

Progesterone and estradiol are the foremost steroid hormones in human pregnancy. However, the origin of maternal progesterone has still not been satisfactorily explained, despite the generally accepted opinion that maternal LDL-cholesterol is a single substrate for placental synthesis of maternal progesterone. The question remains of why the levels of progesterone are substantially higher in fetal as opposed to maternal blood. Hence the role in the fetal zone of fetal adrenal (FZFA) in the synthesis of progesterone precursors was addressed.

The FZFA may be directly regulated by placental CRH inducing an excessive production of sulfated 3β-hydroxy-5-ene steroids such as sulfates of dehydroepiandrosterone (DHEAS) and pregnenolone (PregS). Due to their excellent solubility in plasma these conjugates are easily transported in excessive amounts to the placenta for further conversion to the sex hormones. While the significance of C19 3β-hydroxy-5-ene steroid sulfates originating in FZFA for placental estrogen formation is mostly recognized, the question "Which maternal and/or fetal functions may be served by excessive production of PregS in the FZFA?" - still remains.

It is our hypothesis that, besides the necessity to synthesize *de novo* all the maternal progesterone from cholesterol, it may be more convenient to utilize the fetal PregS. The activities of sulfatase and 3β-hydroxysteroid dehydrogenase (3β-HSD) are substantially higher than the activity of cytochrome P450scc, which is rate-limiting for the placental progesterone synthesis from LDL cholesterol. However, as in the case of progesterone synthesis from maternal LDL cholesterol, the relative independence of progesterone levels on FZFA activity may be a consequence of substrate saturation in enzymes converting PregS to progesterone.

Some of the literature along with our current data (showing no correlation between fetal and maternal progesterone but significant partial correlations between fetal and maternal 20α-dihydroprogesterone (Prog20α) and between Prog20α and progesterone within the maternal blood) indicate that the localization of individual types of 17β-hydroxysteroid dehydrogenase is responsible for a higher proportion of estrone and progesterone in the fetus, but also a higher proportion of estradiol and Prog20α in maternal blood. Type 2 17β-hydroxysteroid dehydrogenase (17HSD2), which oxidizes estradiol to estrone and Prog20α to progesterone, is highly expressed in placental endothelial cells lining the fetal compartment. Alternatively, syncytium, which is directly in contact with maternal blood, produces high amounts of estradiol and Prog20α due to the effects of type 1, 5 and 7 17β-hydroxysteroid dehydrogenases (17HSD1, 17HSD5, and 17HSD7, respectively).

The proposed mechanisms may serve the following functions: 1) providing substances which may influence the placental production of progesterone and synthesis of neuroprotective steroids in the fetus; 2) creating hormonal milieu enabling control of the onset of labor.

Keywords: steroids, progesterone, 20α-dihydroprogesterone, labour, plasma, metabolome, GC-MS

INTRODUCTION

The role of progesterone in the sustaining of human pregnancy is widely known. But the origin of progesterone in the maternal compartment has still not been satisfactorily explained, despite the generally accepted opinion that progesterone is synthesized in the placenta independently of fetal steroids from the cholesterol which is transported from the maternal compartment (Braunstein 2003, Carr and Simpson 1982, Hercz *et al.* 1988, Runnebaum and Rabe 1983). Although this principle conforms with the absence of correlation between maternal and fetal progesterone, there remains a question to be answered : "Why are the levels of progesterone and 5α-dihydroprogesterone (P5α) in the fetal serum substantially higher when compared with the maternal circulation (Antonipillai and Murphy 1977, Farquharson and Klopper 1984, Hercz *et al.* 1988, Kawamura *et al.* 1989, Lofgren and Backstrom 1997, Mathur *et al.* 1980, Runnebaum *et al.* 1975)?" Hence the role of the fetal zone of fetal adrenal (FZFA) in the synthesis of (Donaldson *et al.* 1991) progesterone precursors was addressed.

The FZFA cells lacking 3β-hydroxysteroid dehydrogenase (3β-HSD) may be directly regulated by placental CRH (Smith 2007, Smith *et al.* 1998). Several studies have shown an association between the levels of maternal plasma CRH of placental origin and the timing of parturition (Ellis *et al.* 2002, Hobel *et al.* 1999, Warren *et al.* 1992). CRH is secreted from the placenta predominantly into the maternal blood, but it also enters fetal circulation (Goland *et al.* 1993). The maternal plasma CRH levels increase exponentially as pregnancy advances, peaking at the time of delivery. In women who deliver preterm the exponential increase is rapid, whereas in women who deliver after the estimated date of delivery the rise is slower (McLean *et al.* 1995, Nodwell *et al.* 1999, Torricelli *et al.* 2006). The aforementioned data indicate that CRH stimulation may induce an excessive production of several sulfated 3β-hydroxy-5-ene steroids, including dehydroepiandrosterone sulfate (DHEAS) and pregnenolone sulfate (PregS). While the levels of DHEAS in fetal blood do not exceed the levels of DHEAS in non-pregnant women (Mathur *et al.* 1980, Sulcova *et al.* 1997), the levels of PregS in fetal blood exceed by about 30 times the PregS concentrations in women who are not pregnant (Havlikova *et al.* 2002, Laatikainen *et al.* 1980, Mathur *et al.* 1980).

Production of CRH by the placenta is specific for primates, but only big apes show an exponential rise similar to that in humans. Glucocorticoids stimulate expression of the CRH gene and production of CRH by the placenta. In turn, CRH stimulates the pituitary to produce ACTH, provoking cortisol synthesis in the adrenal cortex. A positive feed-forward loop is formed. The fetal zone of the adrenal glands rapidly involutes after delivery of the placenta, indicating that placental factors, such as CRH, maintain the fetal zone (Smith 2007).

Due to their high polarity, the sulfated 3β-hydroxy-5-ene steroids, like PregS and DHEAS, are excellently soluble in the plasma and may be easily transported in excessive amounts from the FZFA to placenta for further conversion to sex hormones. The levels of PregS in UA are about a hundred times lower than those in unconjugated pregnenolone. Pregnenolone is lipophilic and therefore hardly soluble in plasma. The difference between DHEA and DHEAS levels is even more prominent (Mathur *et al.* 1980).

As reported by Komatsuzaki (Komatsuzaki *et al.* 1987), PregS circulating in maternal blood can be a precursor of various C21 steroids, but due to the absence of cytochrome P45017α in the placenta (Miller 1998, Pepe and Albrecht 1995) it cannot be the precursor of C19 and

C18 steroids. In all probability a similar situation may be expected in the fetus. Obviously, there is a little possibility that fetal PregS may be converted to placental estrogens. The significance of C19 3β-hydroxy-5-ene steroid sulfates, originating in FZFA, for placental estrogen formation determining estrogen levels in both the fetal and maternal compartments is widely recognized (Nodwell *et al.* 1999, Smith 2007, Smith *et al.* 1998), albeit some former reports indicated the source of estrogen synthesis in the maternal compartment (Keresztes *et al.* 1988). Nevertheless, the question of what the excessive production of PregS in the FZFA is good for still remains.

THE HYPOTHESIS

As in a number of physiological processes, in the case of placental progesterone synthesis there may be at least two fungible ways leading to the same goal. Besides the utilization of maternal cholesterol there might be another source for progesterone synthesis depending on the fetal adrenal steroidogenesis and consuming the precursor, which is freely available in fetal circulation, but not in maternal. Instead of synthesizing *de novo* all maternal progesterone from cholesterol, it would be biologically more efficient to utilize PregS amply supplied by FZFA. Placenta freely expresses enzymes necessary for hydrolysis of PregS and conversion of pregnenolone to progesterone (Li *et al.* 2005, Selcer *et al.* 2007). The activity of 3β-HSD in the placenta is substantially higher than that of cytochrome P450scc and is not rate-limiting for placental progesterone synthesis (Boguslawski 1983, Tuckey 2005, Winkel *et al.* 1980). The PregS levels are significantly lower in UVn than those in UA. Alternatively, pregnenolone shows either no difference between sera from the umbilical artery (UA) and umbilical vein (UVn), or a decreasing gradient from UA to UVn being of borderline significance (Kawamura *et al.* 1989, Laatikainen *et al.* 1980, Mathur *et al.* 1980). The former data probably reflect the ready desulfation of PregS in the placenta, while the latter data might indicate saturation of the 3β-HSD capacity by pregnenolone.

However, the considered possibility that maternal progesterone may be partly dependent on fetal steroidogenesis raises the question of how the absence of correlation between maternal and fetal progesterone levels (Farquharson and Klopper 1984, Hercz *et al.* 1988, Oszczygiel 1975) can be explained. The overproduction of the substrate for progesterone synthesis in the fetal compartment overloading the metabolic capacity in the placenta may be the reason why progesterone levels in both compartments depend on the localization of steroid converting enzymes as well as on the transport of steroids within the placenta. But these progesterone levels are independent of the short term fluctuations of steroid levels within the opposite compartment as well as being independent of moderate alterations in substrate (PregS) production. This may be a similar situation to the case of maternal LDL cholesterol being a substrate for the “classical” placental progesterone synthesis. In contrast to the steroidogenesis in maternal adrenal, placental mitochondria have a near-saturating cholesterol concentration for P450scc. So cholesterol translocation to the P450scc is not a major site of the regulation of progesterone synthesis (Tuckey 2005).

According to our hypothesis, progesterone production may be at least partly provided by the most abundant product of the FZFA, PregS. Progesterone originating in the placenta may be selectively distributed into fetal and maternal compartments depending on the permeability of the placental membrane to steroids and the local distribution of 17β-hydroxysteroid dehydrogenases (17β-HSD) determining the balance between progesterone and Prog20α

and, at the same time, the balance between estrone and estradiol. Microsomal type 2 17β-HSD (17HSD2) catalyzes progesterone biosynthesis in the placenta from Prog20α as well as inactivation of estradiol to estrone (Andersson and Moghrabi 1997). The effect of 17HSD2 is countered by types 5 and 7 17β-HSDs (17HSD5 and 17HSD7, respectively) catalyzing progesterone deactivation to Prog20α, while estradiol is synthesized from estrone. Concerning the 17β-hydroxysteroid dehydrogenase type 1 (17HSD1), this cytosolic enzyme shows a high specificity for C18 steroids converting inactive estrone to the active estrogen estradiol.

17HSD2 oxidizing estradiol to estrone and Prog20α to progesterone is highly expressed in placental endothelial cells lining the fetal compartment (Drolet *et al.* 2007, Su *et al.* 2007). On the other side, syncytium, coming directly into contact with maternal blood, produces high amounts of estradiol. Reduction of the low activity estrogen, estrone, into the potent estrogen, estradiol, is catalyzed by 17HSD1. Syncytium is the major steroidogenic unit of the fetal term villi showing immunoreactivities with 17HSD1 mRNA and protein as well as P450scc, P450 aromatase and 3β-HSD. Extravillous cytotrophoblasts (CTBs), e.g. those from which cell columns of anchoring villous originate, also express the 17HSD1 gene. However, CTBs lying beneath the syncytial layer, e.g. those from which syncytiotrophoblasts develop, contained barely detectable amounts of type 17HSD1 mRNA (Bonenfant *et al.* 2000). In contrast to type 17HSD1 mRNA, type 17HSD2 mRNA was not detectable in cell cultures of human cytotrophoblasts or syncytiotrophoblasts. The primary sites of the 17HSD2 gene expression are the endothelial cells of the villous arterioles.

As indicated by the aforementioned findings, the activation of progesterone synthesis by 17HSDs is in all probability closely associated with estradiol catabolism to estrone and vice versa.

This concept is supported by

- higher estrone/estradiol ratios (Kenny *et al.* 1973, Troisi *et al.* 2003) and higher progesterone/Prog20α ratios in fetal circulation (Runnebaum *et al.* 1975) compared with the respective data in maternal blood

- higher progesterone levels in fetal blood compared to maternal venous blood (Antonipillai and Murphy 1977, Farquharson and Klopper 1984, Hercz *et al.* 1988, Kawamura *et al.* 1989, Lofgren and Backstrom 1997, Mathur *et al.* 1980, Runnebaum *et al.* 1975)

- higher estradiol levels in maternal than in fetal circulation (Kenny *et al.* 1973, Troisi *et al.* 2003)

- higher estrone levels in fetal blood in comparison with those reported in maternal blood.

In the case of fetal estrogens the findings may be of physiological importance. As proposed by Drolet and colleagues, 17HSD2 probably protects the fetus from the active estrogen (Drolet *et al.* 2007).

Besides the placenta, the further potent steroidogenic cells expressing 17HSD2 are the hepatocytes (Moghrabi *et al.* 1997). This means that the fetal liver might also influence the balance between estrone and estradiol in both the fetal and maternal compartments. In addition, there are a number of further catabolic pathways, particularly the 16α-hydroxylation and sulfation, which may alter the balance between estrone and estradiol in mother and fetus.

EVALUATION OF THE HYPOTHESIS

When considering the progressively increasing production of CRH, which probably directly stimulates steroid biosynthesis in the FZFA, we can expect higher levels of progesterone in the fetus compared to maternal circulation due to excessive production of PregS synthesized in the FZFA. In accordance with the above mentioned concept, the higher progesterone levels in the human fetus than in the maternal compartment were reported by various authors (Hercz *et al.* 1988, Lofgren and Backstrom 1997, Runnebaum *et al.* 1975). Besides the primates, there are other mammalian species in which progesterone concentrations in fetal circulation are higher compared to the maternal blood (Barnes *et al.* 1975, Hagen *et al.* 1983). From these studies the information reported by Hagen and colleagues may be of importance for placental progesterone biosynthesis in humans. The authors reported that the evident uptake of progesterone from the placenta by fetal blood in pig dams was not equivalent to the maternal uterine arterial-venous difference in progesterone concentration (Hagen *et al.* 1983).

The possibility that maternal progesterone in humans may be synthesized from PregS of the fetal origin is substantiated by the results of Komatsuzaki and coworkers (Komatsuzaki *et al.* 1987). The authors administered deuterated PregS into maternal circulation and they found a certain amount of deuterated progesterone in the cord blood despite the unfavorable concentration gradient. Nevertheless, the penetration of maternal progesterone into the fetal compartment is not very important, as documented by the experiments of Escarcena and colleagues (Escarcena *et al.* 1978) demonstrating that less than 10% of the hormone in fetal circulation is derived from the transfer of maternally circulating progesterone. The authors estimated that the secretion rate of the placental hormone towards the fetus would be about 1/10 of the progesterone secretion rate towards maternal circulation but only about 1% of the maternally circulating hormone was found to cross the placenta. It is obvious that the former findings, as well as the progesterone levels significantly higher in fetal circulation than those ones in maternal blood, contradict the suggestion of an exclusively maternal origin for progesterone in pregnancy.

Some studies indicate an association between the activity of FZFA and placental progesterone production, at least in the fetus. The authors suggested that fetuses exposed to stress during labour produce higher progesterone secretion, which may protect them, i.e. the fetuses, against the sequelae of hypoxia (Antonipillai and Murphy 1977, Shaxted *et al.* 1982). It is likely that the increasing fetal progesterone levels in stressful situations are associated with increased activity of the FZFA producing extreme amounts of PregS.

The association between activity of FZFA and maternal progesterone was also indicated by the data of Sagen and coworkers (Sagen *et al.* 1979), who measured concentrations of total estriol, progesterone, and cortisol in MV at regular intervals from the seventh week of pregnancy until the term in a woman with an anencephalic fetus. Except for the first trimester, during which the values were within the lower normal range, the concentration of estriol was constantly subnormal. Progesterone and HPL were both within the low normal range and the "physiological" rise in cortisol levels was absent. The aforementioned data show that the absence of fetal pituitary results in insensitivity of the definitive zone of fetal adrenal to stress. However, it is apparent that the function of the FZFA is reduced to some extent, but not completely eliminated. This conforms with the concept of direct stimulation of FZFA by placental CRH (Smith 2007, Smith *et al.* 1998). In contrast to Sagen and

colleagues, Kawamura and coworkers reported that the levels of pregnenolone, Preg20α, Preg16α and Prog20α in MV in the 3rd trimester were pronouncedly lower in the case of anencephalic pregnancy than in normal pregnancy, while progesterone levels showed no significant difference. (Kawamura *et al.* 1989). The latter data indicates that the interplay between fetal pituitary and FZFA is important, but placental progesterone production is to a great extent autonomous. The considerable autonomy of placental progesterone synthesis, which might prefer the "classic" mechanism of progesterone synthesis in the case of insufficient PregS availability, was also demonstrated in the studies on anencephalic or dead fetuses (Dawood 1976, MacDonald *et al.* 1982).

Hercz and colleagues (Hercz *et al.* 1988) demonstrated that progesterone production depends on gestational age (GA). The authors reported that the progesterone concentration at labour increased during the 28th - 40th weeks in MV but grows only during 28th - 36th weeks in UVn and UA and then fell significantly by the 40th week. Alternatively, Donaldson *et al.* (Donaldson *et al.* 1991) reported no significant change in the fetal serum levels of progesterone with GA in the samples obtained by transabdominal needling within the 18th and 41st weeks of gestation. In the fetus there was a significant correlation between progesterone and cortisol concentrations. The aforementioned results confirmed high levels of progesterone in the fetus from an early stage of gestation, and provided evidence for placental progesterone being the precursor of fetal cortisol. However, the alternative explanation for the correlation between fetal cortisol and progesterone might be the concurrent effect of CRH on the fetal pituitary and FZFA. Kawamura and colleagues showed that the concentrations of the total 3β-hydroxy-5-ene steroids (including sulfates) in MV progressively increase up to the delivery. Progesterone and Prog20α showed a gradual increase from the 1st trimester to maximum levels at the pre-pain period followed by a rapid decrease at delivery (Kawamura *et al.* 1989). The concurrent dependence of progesterone levels in the maternal and the levels of progesterone and 3β-hydroxy-5-ene steroids in the fetal compartments on GA indicate that there may be relationships between the activity of FZFA and the steroid levels in both compartments although the fluctuations in FZFA activity may not be immediately reflected by changing progesterone levels in maternal compartment.

Concerning our idea about an association between catabolism of progesterone and the biosynthesis of estradiol via the system of placental- and perhaps liver 17β-HSD, a different concept is generally accepted. Shanker and Rao demonstrated that there is a regulating mechanism for progesterone synthesis dependent on estrogen and progesterone receptors (Shanker and Rao 1997). Waddell and colleagues suggest that the estrogen-dependent developmental increase in key components of the progesterone biosynthetic pathway in baboons is associated with a corresponding increase in progesterone production (Waddell *et al.* 1996). The above noted data concerning the estradiol effect on progesterone synthesis, however, may be explained alternatively. FZFA concurrently produces precursors for estrogen and progesterone placental synthesis. Progesterone and estrone reversible inter-conversion to Prog20α and estradiol, respectively, might be catalyzed mostly by the same enzymes.

PRELIMINARY DATA

Subjects

The study group consisted of 50 women (from 21 to 41) in labour from the 28th to the 41st week of gestation. Twelve (24%) women giving birth after the 38th week of gestation were without perinatological complications. From the 38 (76%) labours coming on within the 28th and 37th weeks of gestation, 29 (76.3%) pregnancies were terminated by CS due to health risks to mother or fetus and 9 (23.7%) were vaginal deliveries with spontaneous uterine activity. In the case of these women, the reason for premature uterine activity was infection in the mother. In contrast to the group of healthy women after the 38th week of gestation, all premature births were selected so that the reason for premature uterine activity was independent of steroid status. The local Ethical Committee approved the study. After giving written consent, the patients underwent sample collection.

Sample collection

Samples of blood from UVn and MV were withdrawn immediately after the separation of a newborn from the umbilical cord. Each sample was collected into a cooled plastic tube. The plasma was obtained after centrifugation for 5 minutes at 2000g at 4°C. The samples of plasma were stored at -20°C until analyzed.

Chemicals and reagents

The steroids were purchased from Steraloids (Newport, RI, USA), the Sylon B from Supelco (Bellefonte, PA, USA), the methoxylamine hydrochloride from Sigma (St. Louis, MO, USA) and the solvents from Merck (Darmstadt, Germany).

Instruments

The GC-MS system was supplied by Shimadzu (Kyoto, Japan). The GCMS-QP2010 Plus system consisted of a gas chromatograph equipped with automatic flow control, AOC-20s autosampler and a quadrupole electron-impact detector with an adjustable electron voltage of 10-195 V, which was set-up to a 70 V. A capillary column with a medium polarity RESTEK Rxi (diameter 0.25 mm, length 15 m, film thickness 0.1 µm was used for analyses).

Steroid analysis

The levels of progesterone, 5α/β-dihydroprogesterones and their 20α-hydroxy-metabolites including polar conjugates of 20α-hydroxy-metabolites were obtained in the frame of metabolomic study including 40 unconjugated steroids and 29 steroid polar conjugates measured in the maternal and fetal body fluids using GC-MS. The samples were prepared using the approach reported previously for preparation of methoxylamine-trimethylsilyl derivatives of progesterone and 5α/β-dihydroprogesterones (Hill *et al.* 2007). The polar conjugates of Prog20α were prepared after hydrolysis as described ibidem.

Instrument setup

Electron-impact ionization was used for the analyses. The electron voltage was set up to 70 V and the emission current to 160 μA. The temperatures of the injection port, ion source and interface were maintained at 220°C, 300°C and 310°C, respectively. Analyses were carried out in the splitless mode with a constant linear velocity of the carrier gas (He) maintained at 60 cm/s. The septum purge flow was set up to 3mL/min. Samples were injected using the high pressure mode which was applied at 200 kPa. This pressure was maintained for 1 minute. The detector voltage was set to 1.4 kV.

Temperature and pressure gradients for the GC-MS analysis of trimethylsilyl- derivatives and the retention times of the steroids

To utilize effectively biological material, the individual samples were applied in independent courses employing in each case a part of the steroids under investigation. The choice of the steroids measured within the individual courses, the temperature and the pressure gradients, and the effective masses used for the measurement in selected ion monitoring (SIM) mode were all optimized to attain a minimum limit of detection (LOD) at sufficient selectivity. The temperatures and pressure gradients for the detection of steroids are shown in Table 1. The effective masses, retention times of chromatographic peaks, sequence number of injections for steroid groups and gradients that were used for quantification of individual steroids are shown in Table 2.

Calibration curve

In all cases, the mixtures of authentic standards and internal standard were processed in the same way as samples. The mixtures were specific for each of the independent courses as mentioned above. The standards were injected in duplicates in three different amounts for each steroid (10, 100 and 1000 pg). Respecting the excellent linearity for all substances investigated (the correlation coefficients of two-parameter linear regression ranged from 0.9971 to 0.9999); the calibration line was used for data processing.

Statistical data analysis

To eliminate skewed data distribution and heteroscedasticity, the original data was transformed to a Gaussian distribution and a constant variance before further processing by a power transformation. Relationships between steroid levels were evaluated using Pearson's correlations and partial correlations with an adjustment to constant levels of all the variables in the correlation matrix except for the pair under investigation. The differences between compartments and individual steroids were evaluated using a robust Wilcoxon's paired test. Statistical software Statgraphics Centurion, version XV from Statpoint Inc. (Herndon, Virginia, USA) was used for data analysis.

Results

The levels of progesterone and its 5α/β-3-oxo/20α-metabolites found in the group of 12 women in normal labour within the 38^{th} and 41^{st} weeks of gestation are shown in Table 3. The comparison of the results with the data reported from other studies is provided ibidem (Arai and Yanaihara 1977, Booth and El-Garf 1974, Buster *et al.* 1979, Coats *et al.* 1977, Csapo *et al.* 1971, Gilbert Evans *et al.* 2005, Luisi *et al.* 2000, O'Leary *et al.* 1991, Pearson Murphy *et al.* 2001, Sheehan *et al.* 2005, Soldin *et al.* 2005). The steroid levels generally

agreed with the data reported elsewhere (Buster *et al.* 1979, Gilbert Evans *et al.* 2005, Hill *et al.* 2007, Kawamura *et al.* 1989, Lofgren and Backstrom 1997, Pearson Murphy *et al.* 2001, Runnebaum *et al.* 1975, Sheehan *et al.* 2005). A few discrepancies between our results and results reported previously may be explained by differences in sample collection. Several 20α-hydroxy-C21-steroids and their conjugates were measured in UVn and/or MV for the first time. We have also found no data concerning the levels of P5β in UVn. Progesterone levels were significantly higher levels in UVn than in MV (3.7 times).

As expected, in accordance with the data of a number of the other authors (Farquharson and Klopper 1984, Hercz *et al.* 1988, Mathur *et al.* 1980, Oszczygiel 1975), progesterone levels did not correlate between MV and UVn (Figure 1A, Table 4). On the other hand, all the investigated metabolites of progesterone showed significant associations between MV and UVn (Figure 1B-I, Table 4).

As demonstrated in Table 4, the partial correlations with adjustment to constant levels of all remaining steroids except for the pair of the steroids investigated (below the diagonal) confirmed that progesterone in MV was actually independent of progesterone in UVn. However, both Prog20α and Prog20αC significantly correlated between UVn and MV, while progesterone and Prog20α significantly positively correlated within MV. Similar situation was observed for correlations of P5α, P5β and their respective 20α-hydroxy-metabolites.

CONSEQUENCES OF THE HYPOTHESIS AND DISCUSSION

The above stated data demonstrate that there is an association between maternal and fetal steroids, even if it is not straightforward. Instead of a direct relationship between maternal and fetal progesterone, there is a relatively close association between the compartments for its 20α-hydroxy-metabolite. There are at least two explanations for the partial correlations mentioned above. The findings may indicate that, at least in progesterone and P5α, the parent steroids are primarily converted to their 20α-hydroxymetabolites in the placenta, and then penetrate from the placenta to the fetal and maternal compartments. There they may again be reconstituted to the parent 20-oxo-steroids. In contrast to progesterone and P5α, the P5β may significantly penetrate in the form of the parent 20-oxo-steroid, without primary conversion to P5β20α/C. Nevertheless, even in this case the conversion of P5β to P5β20α/C in the fetus, penetration of the substances into the maternal compartment, and reconstitution of P5β20α/C to P5β herein, are also probable.

The second, and more probable, hypothesis suggesting a different localization of individual 17β-HSDs types in the placenta, which may be simultaneously decisive for Prog20α/progesterone and estrone/estradiol ratios in fetal and maternal compartments, has already been mentioned. As shown in Table 3, the Prog20α levels are similar in fetal and maternal blood, while progesterone levels are almost four times higher in the UVn.

Respecting the aforesaid hypothesis, the results given above are consistent with the situation in estrogens. Estradiol blood levels are about 2.5 times higher in the mother than in the fetus, while estrone levels are about four times higher in fetal blood than in the maternal circulation. This data clearly supports our hypothesis that the localization of 17HSD2 oxidizing estradiol to estrone and Prog20α to progesterone, which is highly expressed in placental endothelial cells lining the fetal compartment (Drolet *et al.* 2007, Su *et al.* 2007), and alternatively syncytium, which is directly in contact with maternal blood and produces high amounts of estradiol due to the effects of 17HSD1, 17HSD5, and 17HSD7 (Andersson

and Moghrabi 1997), are responsible for the higher proportion of the oxidized form of the sex hormones in the fetus, but a higher proportion of the reduced form of these substances in MV.

The consequences of our hypothesis are obvious. The estradiol role in the maternal compartment is widely known. On the other side, the possibility that high progesterone levels in the fetal compartment may provide a substrate pool for the synthesis of neuroprotective 3α-hydroxy-5α/β-pregnane-20-oxo metabolites which in all probability easily penetrate to the fetal brain and may protect the brain neuronal cells from oxidative damage. This hypothesis was quite recently documented by the latest studies (Billiards *et al.* 2006, Hirst *et al.* 2008, Hirst *et al.* 2006, Westcott *et al.* 2008, Yawno *et al.* 2007). The practical consequence of the suggested mechanism may be helpful in the effort to develop the substances, which may influence the placental production of progesterone and, in turn, the synthesis of neuroprotective substances in the fetus, as well as in obtaining the media enabling control of the timing of parturition and the onset of labour.

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Table 1. Temperature gradients used for steroid analysis at constant linear velocity 60 cm·s^{-1}

	Step						
	Initial conditions (final temperature, temperature gradient, hold time)				**Initial pressure**	**Injection Temp.**	**Overall time**
Method	[°C, °C·min-1, min]				[kPa]	[°C]	[min]
G1, G3, GC3	80 (-, 1)	190 (40, 0)	210 (4, 0)	300 (20, 5)	34	220	18.25
G2	80 (-, 0)	190 (40, 0)	205 (1.6, 0)	300 (40, 5)	34	240	19.50

Table 2. Characteristics from analysis of 69 steroids and steroid polar conjugates in the plasma from umbilical artery, umbilical vein and maternal cubital vein and in amniotic fluid at labor from 28th to 41st week of pregnancy

			Retention time [min]		**Peak range for quantitative peak** [min]			
Gradient	**Steroid**	**m/z** [Da]	Peak 1	Peak 2				**σ**[b)]
G1	EpiE2 (IS)	285, 416[a)]	9.97	----	9.91	-	10.02	0.011
G1	P5β	275, 288, 343	11.88	11.90	11.89	-	11.94	0.009
G1	P5α	275, 288, 343	12.15	12.17	12.11	-	12.17	0.008
G2	Prog	273, 286, 372, 341	14.51	14.58	14.47	-	14.54	0.007
G3	EpiE2 (IS)	285, 416	9.97	----	9.92	-	10.02	0.011
G3	P5β20α	288, 303	11.34	11.36	11.35	-	11.42	0.008
G3	P5α20α	288, 303	11.64	11.66	11.65	-	11.71	0.008
G3	Prog20α	153, 296, 301	11.85	11.97	11.81	-	11.88	0.008
G3C	EpiE2 (IS)	231, 285, 416	9.98	----	9.94	-	10.03	0.011
G3C	P5β20αC	288, 303	11.35	11.37	11.36	-	11.42	0.009
G3C	P5α20αC	288, 303	11.65	11.67	11.62	-	11.66	0.009
G3C	Prog20αC	153, 296, 301	11.86	11.98	11.82	-	11.90	0.009

[a)] peaks and effective masses used for quantification are underlined,
[b)] σ…standard deviation of retention time for quantitation peak

Table 3. Comparison of mean values of for the levels (nmol/l) of progesterone (Prog), 5α-dihydroprogesterone (P5α), and 5β-dihydroprogesterone (P5β), their 20α-hydroxymetabolites, 20α-hydroxy-4-pregnene-3-one (Prog20α), 20α-hydroxy-5α-pregnane-3-one (P5α20α), and 20α-hydroxy-5β-pregnane-3-one (P5β20α), and polar conjugates of the 20α-hydroxy-steroids (Prog20αC, P5α20αC, P5β20αC)

Steroid	UVn	MV	Citations
Prog	1440	386	(RIA, w40: MV 478) [Csapo et al., 1971]; (GLC, 3th trimester: MV 239-427) [Booth and El-Garf, 1974]; (GLC, VD: UVn 704±227 MV 129±49) [Runnebaum et al., 1975]; (RIA, w34-40: MV 106-522) [Coats et al., 1977]; (RIA, VD: UVn 1082) [Arai et al., 1977]; (RIA, w40: MV 475) [Buster et al., 1979]; (RIA, VD: UVn 3248, MV 541) [Mathur et al., 1980]; (RIA, CS w37-41: UVn 640, MV 287) [Laatikainen et al., 1980]; (RIA, VD: UVn 585±131, MV 140±28) [Keresztes et al., 1988]; (GC-MS, VD: MV 439, 226 pre pain, delivery) [Kawamura et al., 1989]; (RIA, w40: MV 49-584) [O'Leary et al., 1991]; (RIA, w41: U 822, MV 783) [Donaldson et al., 1991]; (LC-RIA, VD: UVn 1668±1148, MV 156±143) [Lofgren and Backstrom, 1997]; (LC-RIA, VD: MV 478) [Luisi et al., 2000]; (HPLC-RIA, w36-38: MV 659) [Pearson Murphy et al., 2001]; (LC/MS/MS, w32 LP: MV 224) [Soldin et al., 2005]; (GC-MS, w36-38 LP: MV 520) [Gilbert Evans et al., 2005];
Prog20α	50.2	64.4	(GLC, VD: UVn 17±3 MV 15±15) [Runnebaum et al., 1975]; (RIA, w40: MV 79.1) [Buster et al., 1979]; (GC-MS, VD: MV 330, 186 pre pain, delivery) [Kawamura et al., 1989];
Prog20αC	95	35.2	
P5α	36.9	17.6	(LC-RIA, VD: UVn 224±154, MV 82±30) [Lofgren and Backstrom, 1997]; (HPLC-RIA, w36-38: MV 31) [Pearson Murphy et al., 2001]; (GC-MS, w36-38 LP: MV 222) [Gilbert Evans et al., 2005]; (GC-MS, w40 LP: MV 75.6) [Hill et al., 2007];
P5α20α	40.3	28.9	
P5α20αC	90	52.3	
P5β	9.8	1.31	(HPLC-RIA, w36-38: MV 2.3) [Pearson Murphy et al., 2001]; (GC-MS, w36-38 LP: MV 3.54) [Gilbert Evans et al., 2005]; (GC-MS, w40 LP: MV 4.45) [Hill et al., 2007];
P5β20α	13.0	1.65	
P5β20αC	59.2	11.6	

UVn...umbilical vein, MV...maternal cubital vein, U…mixed cord blood, AF…amniotic fluid, VD….vaginal delivery, w…week of gestation (w34-40…34th-40th week of gestation), LP…late pregnancy (not at delivery), CS...Caesarean section

Table 4. Pearson's and partial correlations between 20-oxo and 20α-hydroxy-steroids in sera from umbilical vein (UVn) and maternal cubital vein (MV) for progesterone (Prog), 5α-dihydroprogesterone (P5α), and 5β-dihydroprogesterone (5β) and for their respective 20α-hydroxy metabolites 20α-hydroxy-4-pregnane-3-one (Prog20α), 20α-hydroxy-5α-pregnane-3-one (P5α20α) and 20α-hydroxy-5β-pregnane-3-one (P5β20α); polar conjugates of the steroids are marked by the letter C at the end of the respective abbreviation; simple pair Pearson's correlations and partial correlations with adjustment to constant levels of all steroids in the section except the pair under investigation are above and below the diagonal, respectively.

		Progesterone and its 20α-hydroxy-metabolites					
		UVn			**MV**		
		Prog	**Prog20α**	**Prog20αC**	**Prog**	**Prog20α**	**Prog20αC**
UV	**Prog**		0.199 49 0.170	0.168 48 0.254	0.039 48 0.794	0.095 48 0.519	0.211 48 0.150
	Prog20α	0.147 49 0.335		**0.557** **49** **0.000**	0.268 49 0.063	**0.646** **49** **0.000**	**0.539** **49** **0.000**
	Prog20αC	-0.010 48 0.951	0.211 49 0.164		0.198 48 0.178	**0.527** **48** **0.000**	**0.748** **48** **0.000**
MV	**Prog**	0.042 48 0.785	-0.243 49 0.108	-0.111 48 0.475		**0.679** **48** **0.000**	**0.303** **49** **0.034**
	Prog20α	-0.107 48 0.488	**0.497** **49** **0.001**	0.070 48 0.652	**0.682** **48** **0.000**		**0.617** **48** **0.000**
	Prog20αC	0.137 48 0.374	-0.001 49 0.995	**0.592** **48** **0.000**	-0.050 49 0.745	0.286 48 0.060	
		PARTIAL CORRELATIONS					

		5α-Dihydroprogesterone and its 20α-hydroxy-metabolites					
		UVn			**MV**		
		Prog	**Prog20α**	**Prog20αC**	**Prog**	**Prog20α**	**Prog20αC**
	P5α		**0.677** **48** **0.000**	0.052 48 0.728	**0.519** **49** **0.000**	**0.439** **48** **0.002**	0.047 49 0.750
	P5α20α	**0.612** **48** **0.000**		0.223 48 0.128	**0.582** **49** **0.000**	**0.728** **48** **0.000**	**0.291** **49** **0.042**
	P5α20αC	-0.069 48 0.658	0.093 48 0.548		0.245 49 0.090	**0.338** **48** **0.019**	**0.577** **49** **0.000**
	P5α	**0.378** **49** **0.010**	**-0.301** **49** **0.045**	0.119 49 0.438		**0.820** **49** **0.000**	**0.302** **50** **0.033**
	P5α20α	-0.288 48 0.058	**0.591** **48** **0.000**	-0.096 48 0.537	**0.758** **49** **0.000**		**0.534** **49** **0.000**
	P5α20αC	-0.034 49 0.827	-0.132 49 0.389	**0.500** **49** **0.001**	-0.265 50 0.075	**0.468** **49** **0.001**	
		PARTIAL CORRELATIONS					

		5β-Dihydroprogesterone and its 20α-hydroxy-metabolites					
		UVn			**MV**		
		Prog	**Prog20α**	**Prog20αC**	**Prog**	**Prog20α**	**Prog20αC**
	P5β		**0.682** **48** **0.000**	-0.030 47 0.839	**0.561** **47** **0.000**	**0.385** **47** **0.008**	0.056 48 0.704
	P5β20α	**0.658** **48** **0.000**		0.038 48 0.797	**0.383** **48** **0.007**	**0.619** **48** **0.000**	0.037 49 0.803
	P5β20αC	-0.071 47 0.652	0.036 48 0.818		0.057 48 0.702	0.189 47 0.204	**0.519** **48** **0.000**
	P5β	**0.523** **47** **0.000**	**-0.304** **48** **0.045**	-0.047 48 0.761		**0.563** **48** **0.000**	0.192 48 0.191
	P5β20α	**-0.323** **47** **0.035**	**0.602** **48** **0.000**	0.068 47 0.665	**0.509** **48** **0.000**		0.267 48 0.067
	P5β20αC	0.069 48 0.655	-0.153 49 0.314	**0.495** **48** **0.001**	0.051 48 0.741	0.187 48 0.224	
		PARTIAL CORRELATIONS					

PEARSON'S CORRELATIONS

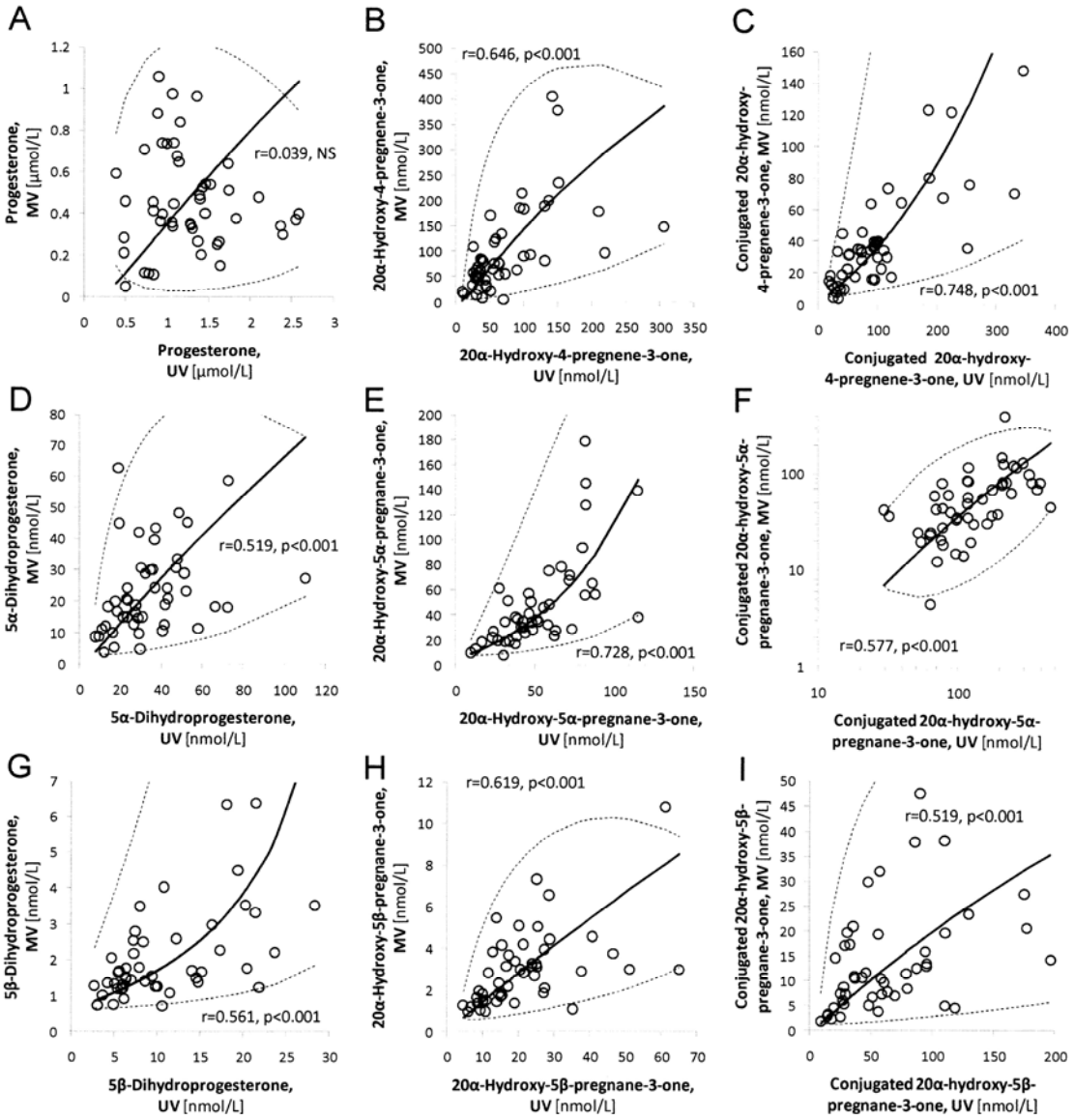

Figure 1: Relationships between steroids in the fetal umbilical venous blood (UVn) and maternal cubital venous blood (MV) for the levels of progesterone (progesterone), 5α-dihydroprogesterone (P5α), and 5β-dihydroprogesterone (P5β), their 20α-hydroxymetabolites, 20α-hydroxy-4-pregnene-3-one (Prog20α), 20α-hydroxy-5α-pregnane-3-one (P5α20α), and 20α-hydroxy-5β-pregnane-3-one (P5β20α), and polar conjugates of the 20α-hydroxy-steroids (Prog20αC, P5α20αC, P5β20αC). The bold full curve represents the principal axis after retransformation to original scale, while the thin dashed line is the retransformed 95% confidence ellipsoid. The correlation coefficient *r* is calculated from the data transformed by a power transformation to attain Gaussian data distribution and a constant variance. For details see Statistical data analysis.