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Review

Steroid metabolome in fetal and maternal body fluids in human late pregnancy

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ABSTRACT

Despite the extensive research during the last six decades the fundamental questions concerning the role of steroids in the initiation of human parturition and origin and function of some steroids in pregnancy were not definitely answered. Based on steroid metabolomic data found in the literature and our so far unpublished results, we attempted to bring new insights concerning the role of steroids in the sustaining and termination of human pregnancy, and predictive value of these substances for estimation of term. We also aimed to explain enigmas concerning the biosynthesis of progesterone and its bioactive catabolites considering the conjunctions between placental production of CRH, synthesis of bioactive steroids produced by fetal adrenal, localization of placental oxidoreductases and sustaining of human pregnancy. Evaluation of data available in the literature, including our recent findings as well as our new unpublished data indicates increasing progesterone synthesis and its concurrently increasing catabolism with approaching parturition, confirms declining production of pregnancy sustaining 5β-pregnane steroids providing uterine quiescence in late pregnancy, increased sulfation of further neuroinhibiting and pregnancy sustaining steroids. In contrast to the established concept considering LDL cholesterol as the primary substrate for progesterone synthesis in pregnancy, our data demonstrates the functioning of alternative mechanism for progesterone synthesis, which is based on the utilization of fetal pregnenolone sulfate for progesterone production in placenta. Close relationships were found between localization of

Abbreviations: 5α-DHT, 5α-dihydrotestosterone; 5β-DHP, 5β-dihydroprogesterone; ACTH, adrenocorticotropic hormone; AF, amniotic fluid; AKRs, aldo-keto reductases; AKR1C1, aldo-keto reductase family 1, member C1, 20α-hydroxysteroid dehydrogenase, hepatic dihydrodiol dehydrogenase; AKR1C2, aldo-keto reductase family 1, member C2, type III 3α-hydroxysteroid dehydrogenase; AKR1C3, aldo-keto reductase family 1, member C3, type II 3α-hydroxysteroid dehydrogenase; AKR1C4, aldo-keto reductase family 1, member C4, type I 3α-hydroxysteroid dehydrogenase; AKR1D1, 5β-reductase; ARSK, arylsulfatase K; CRH, corticotrophin releasing hormone; CRHBP, CRH binding protein; CNS, central nervous system; CYb5, cytochrome b5 enzyme; CYP11A1, cholesterol desmolase, cholesterol side chain cleavage enzyme; CYP11B1, 11β hydroxylase; CYP11B2, aldosterone synthase; CYP17A1, 17α-hydroxylase/17,20 lyase; CYP19A1, aromatase; CYP21A2, P450 21 hydroxylase; CYP3A4, glucocorticoid-inducible P450, taurochenodeoxycholate 6α-hydroxylase; CYP3A5, aryl hydrocarbon hydroxylase, xenobiotic monooxygenase; CYP3A7, aryl hydrocarbon hydroxylase, 16 α-hydroxylase; DHEA, dehydroepiandrosterone; DHEA16α, 16α-hydroxydehydroepiandrosterone; DHEA7α, 7α-hydroxydehydroepiandrosterone; DHEA7β, 7β-hydroxydehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; DZ, definitive zone of the fetal adrenal; FZ, fetal zone of the fetal adrenal; GA, gestational age; $GABA_A$-r, type-A γ-aminobutyric acid receptors; GH, growth hormone; GC–MS, gas chromatography–mass spectrometry; HSD, hydroxysteroid dehydrogenase; HSD11B, 11β-hydroxysteroid dehydrogenase HSD17B1-type 1 17β-hydroxysteroid dehydrogenase; HSD17B10, type 10 17β-hydroxysteroid dehydrogenase; HSD17B11, type 11 17β-hydroxysteroid dehydrogenase, 3(α → β)-hydroxysteroid epimerase; HSD17B12, type 12β-hydroxysteroid dehydrogenase; HSD17B2, type 2 17β-hydroxysteroid dehydrogenase; HSD17B6, type 6 17β-hydroxysteroid dehydrogenase; HSD17B7, type 7 17β-hydroxysteroid dehydrogenase; HSD3Bs, 3β-hydroxysteroid dehydrogenases/Δ(5 → 4)-isomerases; HSD3B1, type I 3β-hydroxysteroid dehydrogenase/Δ(5 → 4)-isomerase; HSD3B2, type II 3β-hydroxysteroid dehydrogenase/Δ(5 → 4)-isomerase; HSDs, hydroxysteroid dehydrogenases; IGF-1, insulin-like growth factor-1; K(m), Michaelis's constant; LC–MS, liquid chromatography–mass spectrometry; LDL, low-density lipoprotein; mRNA, messenger ribonucleic acid; MV, maternal cubital vein; MLN64, steroid acute regulatory protein-related lipid transfer (START) domain containing; NAS, neuroactive steroids; NMDA-r, N-methyl-D-aspartate receptors; OAT-4, organic anion transporter 4; POR, P450 (cytochrome) oxidoreductase; PregS, pregnenolone sulfate; Prog20α, 20α-dihydroprogesterone; SDRs, short-chain dehydrogenases/reductases; SRD5A1, type 1 5α-reductase; SRD5A2, type 2 5α-reductase; StAR, steroid acute regulatory protein; STAT5b, signal transducer and activator of transcription 5B; STS, steroid sulfatase; SULT2A1, human type 2A1 hydroxysteroid sulfotransferase; SULT1E1, estrogen preferring sulfotransferase; TZ, transitional zone of the fetal adrenal; UA, umbilical artery; UGT2B7, UDP glucuronosyltransferase 2B7; UV, umbilical vein.

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placental oxidoreductases and consistently higher levels of sex hormones, neuroactive steroids and their metabolites in the oxidized form in the fetus and in the reduced form in the maternal compartment.

Contents

1. Introduction

Despite the extensive research during the last six decades the questions concerning the role of steroids in the initiation of human parturition and origin and function of some steroids in pregnancy were not definitely answered. Human parturition is unique [1] and therefore the use of animal model is frequently inadequate. Therefore the information contained in steroid metabolome in human body fluids may be helpful for a better understanding the physiology of human pregnancy and parturition. Although the steroid metabolome in maternal circulation was extensively studied, the information is deficient concerning the metabolomic profiles in human fetal body fluids. In this study we attempted to review existing state of art in the steroid metabolomics focused on human late pregnancy. The data found in the literature will be reviewed as well as our so far unpublished GC–MS results that were obtained in the frame of our two recently published studies [2,3] in the group of women at labor from the 28th to 41st week of gestation. The latter data includes almost complete steroid metabolome in fetal umbilical arterial) and venous blood as well as in the maternal venous blood and amniotic fluid. The metabolomic profiles were recorded in 12 women giving birth after the 38th week of gestation who were without perinatological complications and the group of 38 preterm births being selected so that the reasons in preterm labors were independent of the steroid status (for details see [2,3]).

Some of the mechanisms explaining the hormonal control of pregnancy sustaining and onset of parturition involve progesterone withdrawal at concurrently increasing estradiol production before the onset of parturition [4,5]. However, progesterone levels in human maternal blood do not markedly change around parturition [6,7]. Regarding progesterone, the initiation of human delivery is rather connected to a changed expression of specific isoforms of progesterone receptors than to a change in progesterone levels. In addition to the increased circulating estradiol levels, the changing expression of specific isoforms of estradiol receptors probably also contributes to the onset of labor [8]. From the further steroids, cortisol may inhibit progesterone action in the regulation of 15-hydroxyprostaglandin dehydrogenase expression at term [9].

In pregnancy and parturition a role of the most abundant neuroinhibiting reduced progesterone metabolite allopregnanolone was suggested in rats [10]. Allopregnanolone, and probably also some other steroids, operate via positive modulation of the type-A γ-aminobutyric acid receptors ($GABA_A$-r) [11,12] on the membranes of hypothalamic oxytocin-producing cells. However, the role of allopregnanolone and further neuroactive steroids (NAS) in the timing of human parturition is still unclear.

The levels of pregnane NAS are excessively increased in pregnant women [13] in comparison with those in non-pregnant [14]. Besides $GABA_A$-r, the polar conjugates of the reduced 3α/β-hydroxy-5α/β-reduced pregnane steroids are also active on N-methyl-D-aspartate receptors (NMDA-r) showing positive and negative modulation for the 5α- and 5β-isomers, respectively [15]. Although CNS possesses independent steroid production [16], the peripherally produced NAS may pass the blood–brain barrier [17] and influence the steroid metabolome in the CNS. NAS may also operate at the peripheral level like allopregnanolone and progesterone, both attenuating myometrial contractions via the opening of voltage-dependent K^+-channels, contrary to estradiol, which is their antagonist [18,19]. The NAS may be also produced locally, exerting intracrine and paracrine effects. On the other hand, conjugated steroids may be easily transported by circulation in high amounts from more distant sources. The reduced progesterone

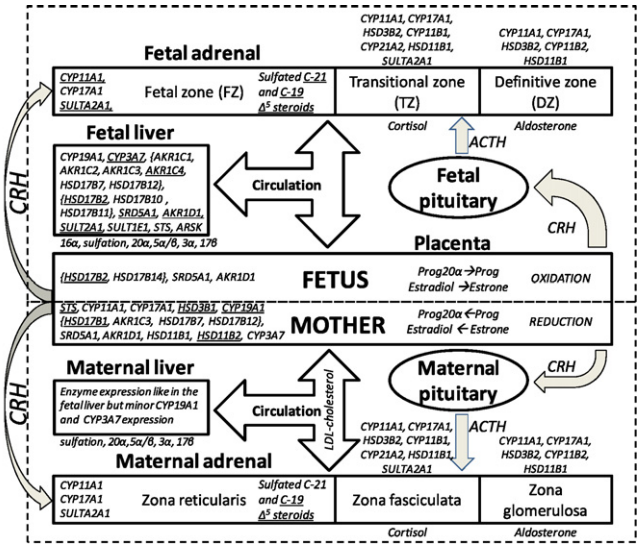

Fig. 1. Simplified scheme of steroidogenesis in human late pregnancy.

metabolites might also exert peripheral analgesic effects via blockade of T-type calcium channels, which are responsible for pain perception [20].

Besides non-genomic effects, reduced progesterone metabolites, which are synthesized in large quantities in pregnancy [13,21–27], may also bind on nuclear receptors such as progesterone receptors [28] providing uterine quiescence. Some studies including ours reported decreasing production of pregnancy sustaining 5β-pregnane steroids that provide uterine quiescence via binding to nuclear pregnane X-receptors [13,26,29–31].

The kinetics of irreversible catabolism of the bioactive steroids, oxidoreductive balances between active and inactive forms of steroids [32] and balances between free steroids and their conjugates [33] may be crucial for the regulation of their biological activity and consequently for the pregnancy sustaining.

Concerning the steroid metabolome in human body fluids, there are four key steroidogenic organs such as fetal and maternal adrenal, placenta, fetal and maternal liver (Fig. 1, our so far unpublished data). Considering the endocrine, autocrine and paracrine steroid effects, the uterus and fetal membranes might be of a great importance [34]. However, the contribution of the steroids produced in these tissues to steroid metabolome in fetal and maternal blood does not seem to be essential.

2. Steroid metabolism in fetal and maternal adrenal

2.1. The key role of placental CRH in the regulation of steroid biosynthesis in pregnancy

The paramount mechanism controlling overall production of the most of pregnancy steroids is based on placental production of CRH (Fig. 1, our so far unpublished data) [35]. CRH in non-pregnant subjects is a hypothalamic hormone controlling the pituitary secretion of ACTH and, in turn, the production of corticosteroids in an adult adrenal. The hypothalamic–pituitary–adrenal axis in these subjects is based on a negative feedback loop between the final active hormone, ACTH and CRH. The situation in pregnancy after luteo-placental shift is different. CRH is primarily expressed in human placenta and instead of the negative feedback loop cortisol–ACTH–CRH; there is a positive one between cortisol and CRH, while the ACTH production stagnates. CRH directly stimulates production of Δ^5 steroid sulfates in the fetal zone of the fetal adrenal (FZ) [36,37] and cortisol synthesis in the transitional zone of the fetal adrenal (TZ) [38] via binding to ACTH receptors [39]. ACTH receptor mRNA is localized in all cortical zones but its abundance is higher in DZ (definitive zone) than in FZ [40]. The fetal adrenal gland at term is almost the size of the fetal kidney and the FZ at term produces steroids more abundantly than normally secreting adrenal glands of the adult [35]. The C-19- and possibly also the C-21 Δ^5 steroids, originating in the FZ and being further processed in placenta and liver, represent the largest fraction of steroids in pregnancy [41–44]. However, progesterone is commonly considered to originate mainly in placenta from maternal LDL cholesterol [7,45].

After midgestation, the TZ cells may have the capacity to synthesize cortisol and be analogous to cells of the *zona fasciculata* of the adult adrenal. By the 30th week of gestation, the definitive zone of the fetal adrenal (DZ) and TZ begin to resemble the adult *zona glomerulosa* and *zona fasciculata*, respectively [46]. The FZ still producing conjugated C-19 Δ^5 steroids is similar to the adult *zona reticularis* but unlike the adult *zona reticularis*, the FZ produces excessive amounts of conjugated C-21 Δ^5 steroids, including sulfates of pregnenolone (PregS), 17-hydroxypregnenolone [35] and androstenediol (Fig. 2, our so far unpublished data). As generally accepted, the Δ^5 steroid sulfates (originating in the FZ) serve as precursors for the placental production of estradiol [36,37] and as suggested in our recent study [2], possibly also for progesterone synthesis.

The levels of CRH are extremely high in maternal and high in the fetal blood [47]. The rising levels of human placental CRH in maternal circulation in the last 4 weeks of pregnancy stimulate the production of conjugated C-19- [36] and probably also the C-21 Δ^5 steroids [2] in FZ in a dose-dependent manner. CRH is as effective as ACTH at stimulating sulfated dehydroepiandrosterone (DHEAS) production but is 70% less potent than ACTH at stimulating cortisol production. Although CRH increases the expression of cholesterol desmolase (CYP11A1, cholesterol side chain cleavage enzyme) it is not mitogenic for fetal adrenal cortical cells [36].

It should be outlined that the excessive production of placental CRH is specific for primates and the boost in CRH production in late pregnancy is specific only for human and great apes [48]. This should be considered when addressing the initiation of human parturition and this is the primary reason for which the animal models may not be optimal for investigation of human pregnancy. Only human beings and great apes produce a circulating binding protein for CRH (CRHBP), the levels of which fall at the end of pregnancy thus increasing the bioavailability of CRH [49,50].

Despite the substantial alterations in the placental CRH production in late pregnancy, the predictivity of the unstable CRH for an estimation of term is relatively poor [51]. Nevertheless, the CRH induced changes in the steroid metabolome may better predict the approaching parturition. When using the simultaneous quantification of the steroid metabolome in one sample by GC–MS or LC–MS and multivariate approach for evaluation of the results obtained, the cumulative effect of mutually strongly inter-correlated steroids substantially improves the predictivity. This algorithm appears to be less expensive and more informative. As demonstrated in our recent study [3], the predictivity of the primary products of the FZ for the onset of human parturition is high.

2.2. Steroid 17α-hydroxylase/17,20 lyase (CYP17A1)

Besides stimulation of CYP11A1, CRH also stimulates 17α-hydroxylase/17,20 lyase (CYP17A1) expression possessing both 17α-hydroxylase and 17,20-lyase activities [36]. CYP17A1 proteins and mRNAs were detected only in FZ and TZ, not in the DZ [52,53]. CYP17A1 also exhibits marked progesterone 16α-hydroxylase activity in human steroidogenic cells including those from the fetal adrenal [54]. CYP17A1 has extremely low C-17,20-

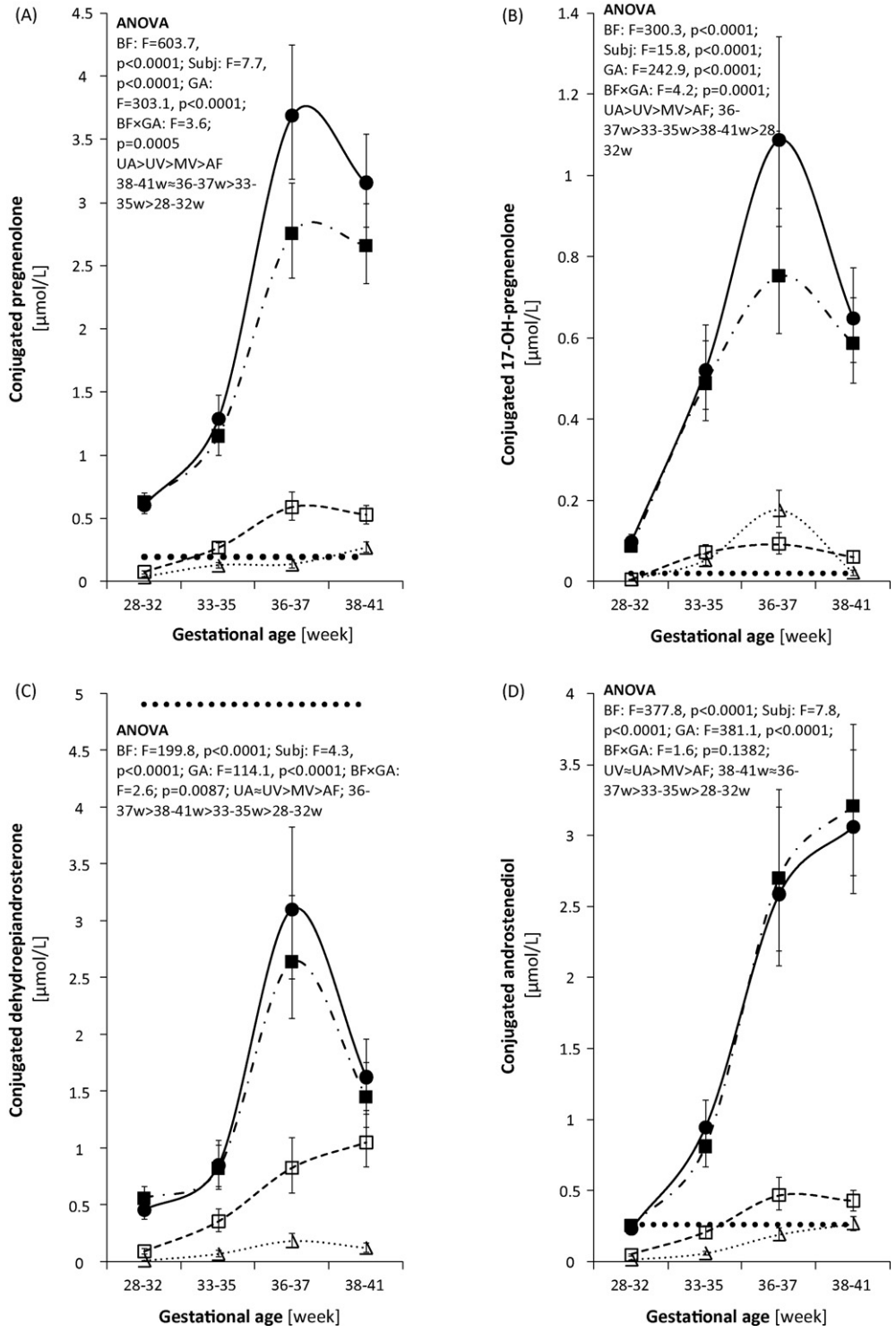

Fig. 2. Profiles of conjugated sulfated Δ^5 steroids in the plasma from the umbilical artery (UA), umbilical vein (UV) and maternal cubital vein (MV) and in amniotic fluid (AF) according to the gestational age. The repeated measures ANOVA model was used for the evaluation of the relationships between steroid levels, GA and the type of body fluid. The model consisted of within-subject factor body fluid (factor BF—four body fluids were investigated in each subject), subject factor (factor Subj), between-subject factor gestational age (factor GA—the subjects were separated into 4 groups according to the GA) and body fluid × GA interaction (BF × GA interaction). The symbol w denotes the week of gestation. Significant BF × GA interaction indicates that there is a significant difference between the dependences of the individual body fluids on GA. *F*-ratio represents the Fisher's statistic and *p* designates statistical significance for the factors and interaction. The symbols with error bars represent re-transformed means with their 95% confidence intervals for individual body fluids (full circles, UA; full squares, UV; empty squares, MV; empty triangles, AF. The significance testing in the form of the subgroup confidence intervals is for the interaction of body fluid (sample material) with GA. The 95% confidence intervals are computed using the least significant difference multiple comparisons ($p < 0.05$). The confidence intervals, which do not overlap each other, denote significant difference between the respective subgroup means. Further embedded table contains the multiple comparisons that are completed separately for the gestation week and for the sample material (body fluid). The symbol "≈" expresses insignificant difference, while the symbol ">" means "significantly higher than". The significance level was considered for $p < 0.05$. The horizontal line from the full circles represents the mean level of the steroid in the luteal phase of the menstrual cycle. These so far unpublished data were obtained in the frame of our recently published studies [2,3].

lyase activity toward C-21 Δ^4 steroids and fails to convert these substances to corresponding C-19 steroids [54]. However, also the levels of sulfated C-21 Δ^5 steroids are elevated in the maternal blood [55] and excessively elevated in the fetal circulation in contrast to the situation in non-pregnant women (Fig. 2, our so far unpublished data), which indicates limited C-17,20-lyase activity in the FZ also for C-21 Δ^5 steroids.

2.3. Steroid sulfotransferases and sulfatases

The human type 2A1 hydroxysteroid sulfotransferase (SULT2A1) displaying reactivity towards 3α/β-hydroxysteroids, estrogens, and 17-hydroxyl group of androgens is highly expressed in the adrenal cortex [56]. TZ and FZ showed immunoreactivity for SULT2A1, but not the DZ [53]. SULT2A1 enzyme activities are independent of the gestational age (GA) [57]. In addition to the SULT2A1 expression, the estrogen preferring sulfotransferase (SULT1E1) activity [58] and relatively high steroid sulfatase (STS) immunoreactivity were also reported in the adult adrenal gland [59].

2.4. Activities of enzymes enrolled in the synthesis of corticoids

3β-Hydroxysteroid dehydrogenases/$\Delta(5\rightarrow4)$-isomerases (HSD3Bs) catalyzes the oxidative conversion of 3β-hydroxy-Δ^5 steroids. HSD3B immunoreactivity is not detected in the fetal adrenal prior to 22 weeks of gestation, but becomes discernible in the TZ and DZ after 23 weeks [52,53]. In late pregnancy, TZ and DZ provide the conversion of Δ^5 steroids to 3-oxo-Δ^4 precursors of corticosteroids expressing type 2 HSD3B (HSD3B2) [60]. Early in gestation, only the Δ^5 steroid production occurs in the TZ and FZ, which expresses CYP11A1 and CYP17A1 [52]. ACTH does not influence steroidogenesis in the FZ [61]. Like the HSD3B2, the enzymes CYP21A2 (P450 21 hydroxylase, or P450C-21), CYP11B1 (11β hydroxylase or P450c11) and CYP11B2 (aldosterone synthase) are necessary for corticoid synthesis. CYP21A2 immunoreactivity is minor in the DZ but is detectable in almost all cells in the TZ and FZ [53,62]. After 23 gestational weeks, the immunoreactivity for CYP21A2 is detected in all three zones [53]. TZ expressing CYP11A1, CYP17A1, HSD3B2, CYP21A2, CYP11B1 and type CYP11B2 has the capacity to synthesize cortisol after midgestation [53,62] while the DZ may synthesize mineralocorticoids, but not until near term [62]. CYP17A1, CYP11B1, and CYP11B2 immunoreactivities are present in the TZ and FZ but absent in the DZ but [62]. Later in gestation, the DZ produces mineralocorticoids, TZ produces glucocorticoids and the FZ continues to produce Δ^5 steroids [52].

Human adrenal glands also possesses 11β-hydroxysteroid dehydrogenase (HSD11B) activity catalyzing inactivation of glucocorticoids [63].

2.5. Adrenal C-3, C-17 and C-20 oxidoreductive conversions

HSDs, catalyzing reversible C-3, C-17 and C-20 oxidoreductive inter-conversions belong to either the short-chain dehydrogenases/reductases (SDRs) or the aldo-keto reductases (AKRs). Several SDRs are active in the adrenals.

The type 11 17β-HSD (HSD17B11) $3(\alpha\rightarrow\beta)$-hydroxysteroid epimerase prefers the oxidative conversion converting 5α-androstan-3α,17β-diol to androsterone [64,65].

Type 6 17β-HSD (HSD17B6) possessing both oxidoreductase and $3(\alpha\rightarrow\beta)$-hydroxysteroid epimerase activities acts on both C-19 and C-21 3α-hydroxysteroids. Because bioactive steroids commonly exert their effect in a stereo-specific manner, epimerase activity may be of biological importance [66].

Type 7 17β-HSD (HSD17B7) preferably operates as reductase and catalyzes the reduction of the oxo-group in either 17- or 3-position of the substrate to the corresponding 17β- or 3α-hydroxy-counterparts, respectively. HSD17B7 exhibits also minor 3βHSD-like activity towards progesterone and 20α-dihydroprogesterone (Prog20α) [67]. Like the HSD17B7 the type 12 17β-HSD (HSD17B12) also prefers the reductive direction catalyzing the conversion of estrone into estradiol and was also detected in the adrenal [68].

2.6. 16α-Hydroxylation

16α-Hydroxylation being primarily provided by cytochrome P450 CYP3A7 enzyme probably regulates the levels of precursors for the synthesis of hormonally active steroids. The CYP3A7 is also active in the fetal adrenal but the levels of the CYP3A7 isozyme in fetal adrenals are only 33% of that in fetal livers [69,70].

2.7. Differences in enzyme expression between fetal and adult adrenal

While the expression of some enzymes like CYP17A1, 21-hydroxylase, 11β hydroxylase, and CYP11B2 do not significantly differ between the fetal adrenal in late pregnancy and adult adrenal, others show pronounced differences. CYP11A1, cytochrome b5 enzyme (CYb5) and P450 cytochrome oxidoreductase (POR) mRNA expression is nearly twice higher in fetal than in adult adrenal, and SULT2A1 transcript shows even 13-fold higher levels in the fetal adrenal. Alternatively, HSD3B2 mRNA expression in midgestation is 127-fold lower than that in the adult adrenal. It is evident that increased expression of CYP11A1 in fetal adrenal reflects high cholesterol utilization for steroidogenesis. CYb5 and POR cofactors may stimulate CYP17A1 activity and thus the production of sulfated Δ^5 steroids in the fetal adrenal [71]. Markedly higher expression of SULT2A1 reflect high claim for steroid sulfation enabling a production of sufficiently soluble precursors, which can be easily transported in excessive amounts by circulation for the placental synthesis of sex hormones. Alternatively, the lack of HSD3B2 in the FZ provides preferential synthesis of the Δ^5 C-21 steroids over cortisol production.

3. Steroid metabolism in fetal and maternal liver

The activities of CYP11A1 and HSD3Bs in the fetal liver are negligible or even absent in human pregnancy [72]. However, other steroidogenic enzymes in the maternal and particularly in the fetal liver may substantially influence the steroid metabolome in both fetal and maternal circulation.

3.1. Liver 16α-hydroxylation and estrogen formation

16α-Hydroxylation is provided by cytochrome P450 CYP3A7 enzyme that is pronouncedly expressed in the microsomal fraction from fetal liver [70,73] although this activity in the adult liver is negligible [43]. While CYP3A4 and CYP3A5 enzymes are responsible for the production of 7α-hydroxy-DHEA (DHEA7α), 7β-hydroxy-DHEA (DHEA7β), and 16α-hydroxy-DHEA (DHEA16α) in the adult liver microsomes, the fetal/neonatal CYP3A7 produces DHEA16α and DHEA7β [74].

The fetal liver is the primary source of 16α-hydroxy-metabolites of Δ^5 steroids, as also documented by consistently higher levels of 16α-hydroxy-metabolites of the substances in fetal circulation when compared with the maternal compartment [75,76], confirmed also by our unpublished results (Fig. 3). However, some authors [54,75] suggested 16α-hydroxy-progesterone (Prog16α) synthesis from progesterone catalyzed by CYP17A1 localized in the placenta.

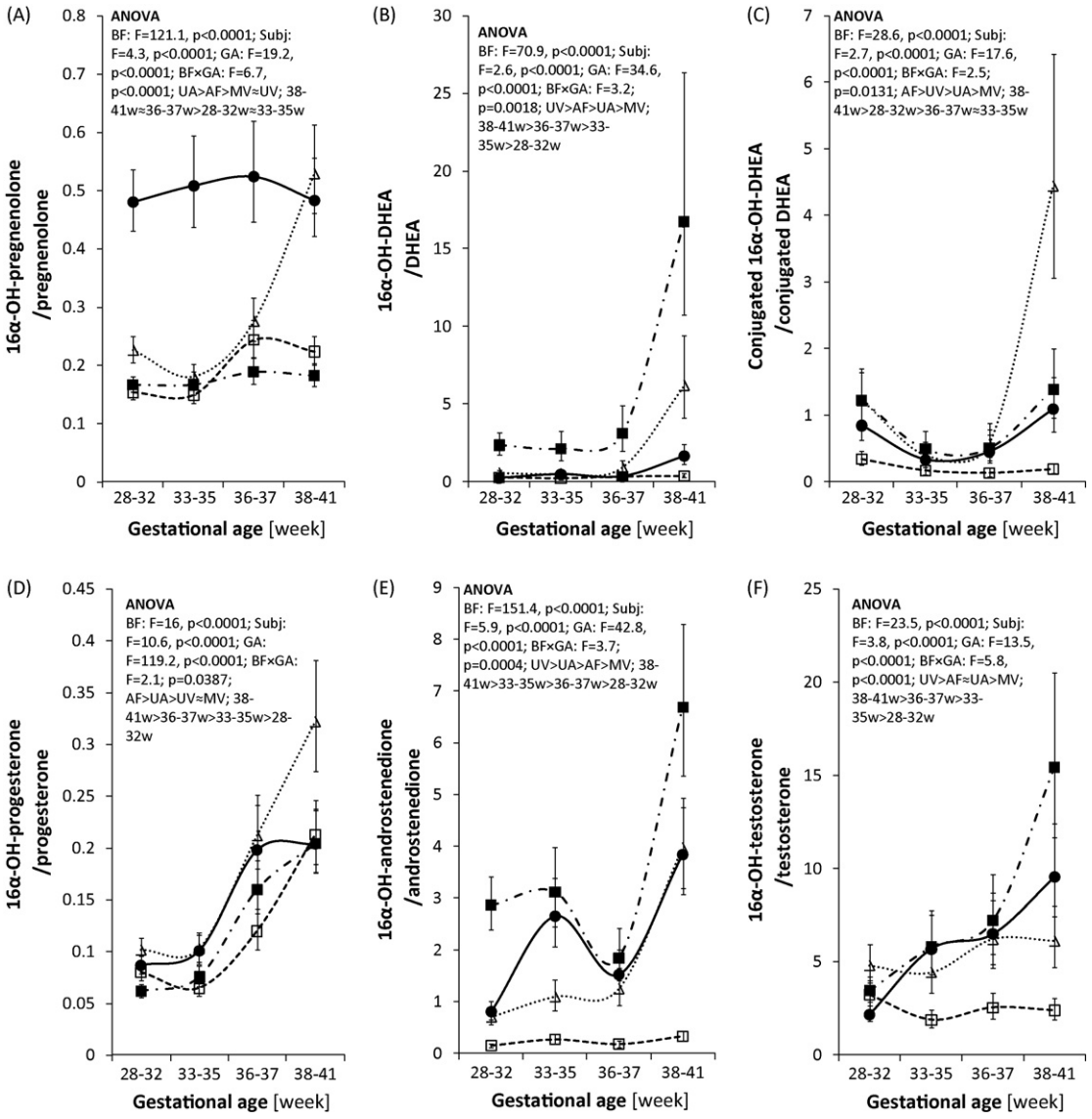

Fig. 3. Profiles of the ratios of 16α-hydroxysteroids to the corresponding 16-deoxy-steroids in the plasma from the umbilical artery (UA), umbilical vein (UV) and maternal cubital vein (MV) and in amniotic fluid (AF) in preterm and normal labor. The drawings and symbols are the same as for Fig. 2. These so far unpublished data were obtained in the frame of our recently published studies [2,3].

The levels of 16α-hydroxysteroids in the fetal blood increase from the second to the third trimester [61,75,76] and rise considerably at delivery [77]. In addition, the ratios of 16α-hydroxy-metabolites to 16-deoxy-steroids significantly increased after 30th week of gestation indicating increasing catabolism of the sex hormone precursors in the fetal liver [78] (Fig. 3, our so far unpublished data).

According to the Diczfalusy's concept [79], the DHEAS from the fetal adrenals is hydroxylated at the 16α-position in the fetal liver and then aromatized to estriol in the placenta and most of this huge amount of estriol exits the placenta into the uterine vasculature and maternal circulation.

The inhibitory effect of sulfated DHEA16α on estrogen production is minimal at low DHEAS concentrations (favoring the secretion of estrone and estradiol) and is greatly enhanced at concentrations of DHEAS that induced maximum estrone and estradiol secretions. In trophoblastic cells, the metabolism of DHEAS can modulate estriol secretion, and the metabolism of sulfated DHEA16α can modulate the secretion of estrone and estradiol [80]. However, whilst each substrate appeared to inhibit the aromatization of the other, the 16-deoxy-C-19 steroids are more potent inhibitors [81]. 16α-Hydroxy-metabolites of testosterone and androstenedione are only poor substrates for the placental aromatase (CYP19A1) in contrast to the corresponding 16-deoxy-steroids [82] and the initial rates of estrogen formation are higher for the 16-deoxy-C-19 steroids [81].

Lee et al. [83] reported that at a physiologically relevant low substrate concentration (10 nmol/L), CYP3A7 had a strong catalytic activity for the 16α-hydroxylation of estrone, and the ratio of its 16α-hydroxylation to 2-hydroxylation was 107%. However, when estradiol was the substrate, CYP3A7 had only very weak catalytic activity for 16α-hydroxylation, and the ratio of its 16α-hydroxylation to 2-hydroxylation was 10–33%. Moreover, the maximum velocity/K(m) ratio was more than 100 times higher for the 16α-hydroxylation of estrone than for estradiol. This prompts that estrone originating in placenta from androstenedione is transported by circulation into the fetal liver, where may be further conjugated by sulfatases and glucoronidases, converted to estradiol by reductive and SDRs and AKR1Cs. Estradiol of the placental

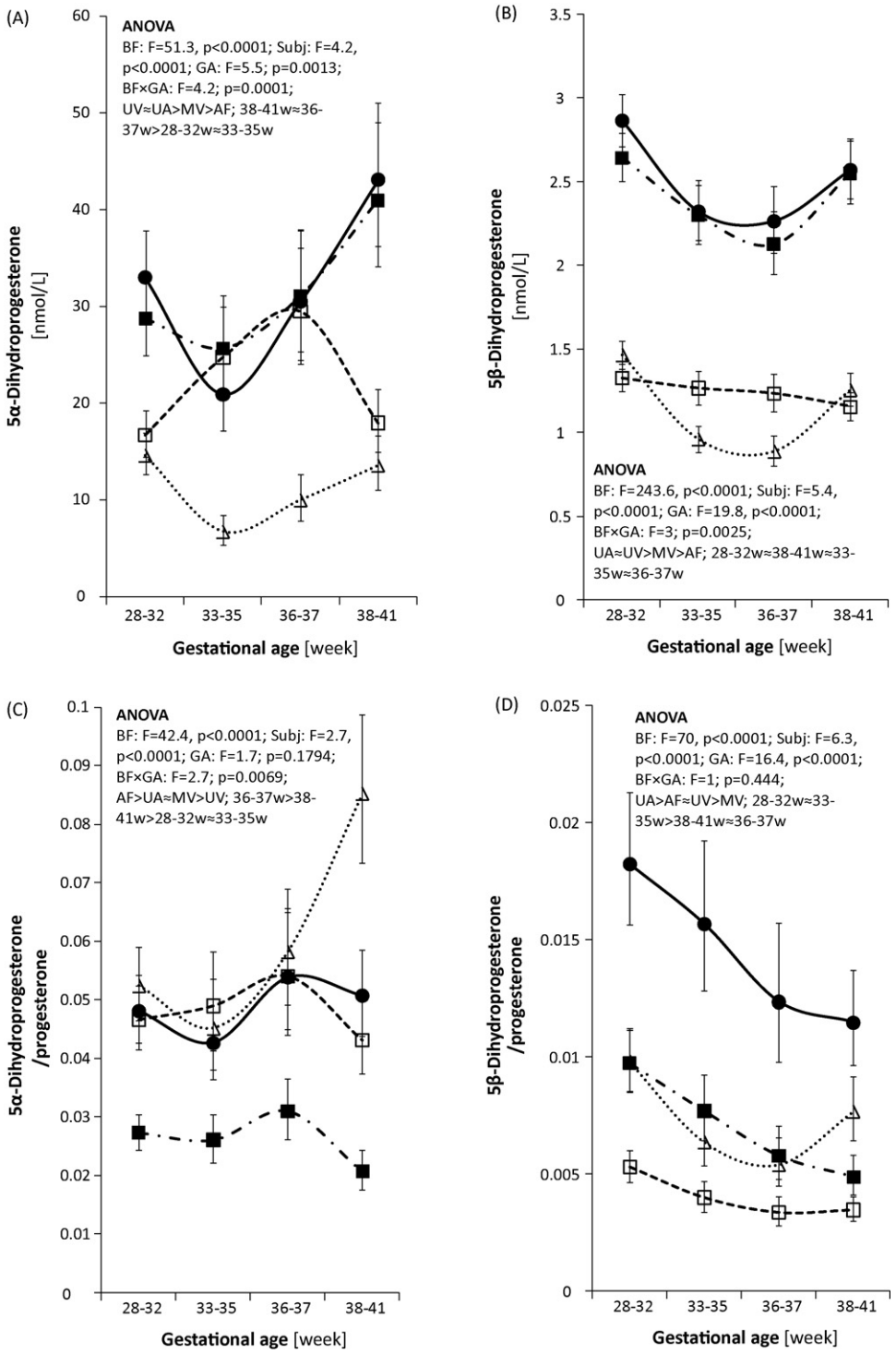

Fig. 4. Profiles of 5α-dihydroprogesterone and 5β-dihydroprogesterone and their ratios to progesterone in the plasma from the umbilical artery (UA), umbilical vein (UV) and maternal cubital vein (MV) and in amniotic fluid (AF) in preterm and normal labor. The drawings and symbols are the same as for Fig. 2. These so far unpublished data were obtained in the frame of our two recently published studies [2,3].

and liver origin may be also sulfated, then the free and conjugated estrone may be 16α-hydroxylated by CYP3A7 (the most potent AK1C4 is independent of the substrate sulfation status) and finally, liver SDRs and AKR1Cs may catalyze further conversion of the free and conjugated (in C-3 position) 16α-hydroxy-estrone to free and conjugated estriol.

Like in the case of CYP3A7, the adult liver exhibits little CYP19A1 activities but the fetal liver is capable to extensively aromatize various C-19 steroids to estrogens [84,85] and the CYP19A1 activity in the fetal hepatocytes appears to be up regulated by glucocorticoids [86].

3.2. C-3, C-17 and C-20 oxidoreductive conversions

Because active hydroxysteroids generally exert their effect in a stereo-specific manner, epimerase activity may potentially play an important role in regulating the biological activities of various steroids.

Human liver contains all isoforms (AKR1C1–AKR1C4) of dihydrodiol dehydrogenase with 20α-, 17β-, 3α- or 3β-hydroxysteroid dehydrogenase-like activity [87–89]. Activities of AKR1Cs could control occupancy of the androgen- and $GABA_A$-r [90]. *In vivo*, all AKR1Cs preferentially work as reductases [91] and are capable to reduce estrone and progesterone to estradiol and Prog20α, respectively. On the other hand, AKR1Cs may decrease the neurosteroid concentrations by inactivating allopregnanolone and eliminating the precursors like progesterone from the synthetic pathways via reduction of the 20-oxo-steroid group [32,92]. The AKR1C2 preferring 3α-reduction over the 3β-reduction may catalyze 3α-, 17β- and 20α-HSD reactions [32,89,92,93]. AKR1C3 catalyze the reduction of 5α-dihydrotestosterone (5α-DHT), androstenedione, estrone and progesterone to produce 5α-androstan-3α,17β-diol, testosterone, estradiol and Prog20α, respectively [88]. AKR1C4, the expression of which is limited to the liver [32,94,95], catalyzes the transformation of the 5α-DHT into 5α-androstan-3α,17β-diol. Liver specific AKR1C4 shows superior catalytic efficiency versus the other isoforms. This efficiency exceeded those obtained with the other isoforms by 10–30-fold. In contrast to the other isoforms, the catalytic efficiency for AKR1C4 is unaffected by steroid conjugation [89].

Two liver SDRs, HSD17B7 and HSD17B12, also preferentially work as reductases. HSD17B7 preferring the reduction of the oxo-group in 20-, 17- or 3-position to the corresponding 20α-hydroxy-, 17β-hydroxy- or 3α-hydroxy-counterparts is also significantly expressed in the liver [67,96] as well as HSD17B12 catalyzing the transformation of estrone into estradiol [68].

HSD17B2, HSD17B10 and HSD17B11, which are also highly expressed in the liver, prefer the oxidative direction. HSD17B2 may contribute to formation of 20-oxo- and 17-oxo-steroids from their 20α- and 17β-counterparts [42]. HSD17B6 prefers oxidoreductase and 3(α → β)-hydroxysteroid epimerase activities and acts on both C-19 and C-21 3α-hydroxysteroids [66]. Type 10 17β-HSD (HSD17B10) being abundantly expressed in the liver, is capable of catalyzing the oxidation of steroid modulators of $GABA_A$-r [97]. HSD17B10 catalyzes the oxidation of 5α-androstan-3α,17β-diol to 5α-DHT [98] and conversion of allopregnanolone and allotetrahydrodeoxycorticosterone (3α,5α-THDOC) to the corresponding inactive 3-oxo-steroids. The catalysis of HSD17B10 appears to be essential for maintaining normal functions of GABA-ergic neurons [99]. Finally, the HSD17B11 [64] can convert 5α-androstan-3α,17β-diol to androsterone [64,65].

Commonly, 20α-hydroxysteroids are considered as inactive catabolites. However, 20α-dihydropregnenolone relax the tonic contractions induced by KCl in a concentration-dependent way [100].

3.3. 5α/β-Reductases

5α- and 5β-Reductions are important for the biosynthesis of NAS. Conjugation of the androgens occurs extensively in the liver which has high activity of 5α- and 5β-reductases [101,102].

There are two isoforms of 5α-reductase, with a limited degree of homology, different biochemical properties and distinct tissue distribution. Type 1 5α-reductase (SRD5A1) is widely distributed in the body, with the highest levels in the liver. SRD5A1 converts testosterone into 5α-dihydrotestosterone and progesterone or corticosterone into their corresponding 5α-3-oxo-steroids. In the androgen-dependent structures, 5α-DHT is almost exclusively formed by 5α-reductase type 2 (SRD5A2) [103]. In the peripheral tissues, including the liver, SRD5A1 and reductive 3α-HSD isoforms work consecutively to eliminate the androgens and protect against the hormone excess [104].

5β-Reductase (AKR1D1) belonging to AKRs, efficiently catalyzes the reduction of both C-19 and C-21 3-oxo-Δ^4 steroids to the corresponding 5β-reduced metabolites. 11β-Hydroxy-group in corticoids hinders the transformation [102].

The higher levels of several 5β-reduced progesterone metabolites in the fetus than in maternal compartment (Figs. 4B, D and 5C, D) (our unpublished data) indicate higher placental expression of AKR1D1 towards the fetal compartment and/or higher expression of AKR1D1 in the fetal liver. The latter possibility appears to be more likely because both 5β-pregnanolone isomers display lower levels in the blood from umbilical vein (UV) than in blood from the umbilical artery (UA) (Fig. 5C and D, our so far unpublished data). In addition, the ratio of 5β-dihydroprogesterone (5β-DHP) to progesterone is significantly higher in UA than in UV (Fig. 4D, our so far unpublished data).

3.4. Balance between polar conjugates and unconjugated steroids

The balance between the sulfated and unsulfated NAS may be decisively influenced by the activities of liver sulfatases, sulfotransferases and perhaps also the glucuronosyltransferases. The pregnane and androstane 5α/β-reduced metabolites being frequently neuroactive are readily sulfated in the liver. As already mentioned, the balances between free steroids and their conjugates [33] may be crucial for the regulation of their biological activity and consequently for the sustaining of pregnancy. The 5α/β-reduced metabolites with a hydroxyl in the 3α-position positively modulate $GABA_A$-r. Their sulfates operate in the opposite way, though on different binding sites. Sulfation may also decrease the concentration of unconjugated NAS, the polarity of which is more favorable for crossing the blood–brain barrier. The modulation efficiencies of the conjugated neurosteroids on $GABA_A$-r may reach about 1/10 of those for the corresponding unconjugated substances [33]. Nonetheless, in maternal circulation the concentrations of conjugated pregnane steroids are about two orders of magnitude higher when compared with their unconjugated analogues. Conjugation is a prerequisite for the activity of 3α/β-hydroxy-5α/β-reduced pregnane steroids on N-methyl-D-aspartate receptors (NMDA-r) showing positive and negative modulation for the 5α- and 5β-isomers, which are neuroactivating and neuroinhibiting substances, respectively [15]. Finally, the sulfation might influence the activity and/or availability of the peripherally active pregnancy sustaining steroids like the 5α/β-reduced pregnane and androstane steroids but may also facilitate their transport by circulation. However, even in these cases, the sulfation rather shift the biological activity towards induction of labor, catabolizing the 5β-reduced steroids that provide uterine quiescence via pregnane X-type receptors [29] and allopregnanolone that relaxes myometrium through voltage-dependent K^+ channels [105].

Our previous [13] and current data consistently show rising sulfation of all pregnanolone isomers including neuroinhibiting GABA-ergic substances in late pregnancy (Fig. 5E–H, our so far unpublished data).

The sulfotransferase SULT2A1 is highly expressed in human liver [56,106–108]. In the fetal liver, SULT2A1 activity exhibits remarkable inter-individual variability, which may be the cause for an absent correlation with the GA [57]. Liver UDP glucuronosyltransferase 2B7 (UGT2B7) catalyzes the glucuronidation of bile acid substrates but also the 3α-hydroxylated androgenic steroids, and 17β-estrogens at very high rates [109].

The sulfotransferase enzyme SULT1E1 has the lowest K(m) values for estrogens and catecholestrogens of the known human SULT isoforms [110]. SULT1E1 is responsible for the sulfation and inactivation of estradiol at physiological concentrations. The enhanced SULT1E1 activity may have a role in inhibiting GH-stimulated STAT5b phosphorylation and IGF-1 synthesis via the sulfation and inactivation of estradiol [111]. SULT1E1 may also play an impor-

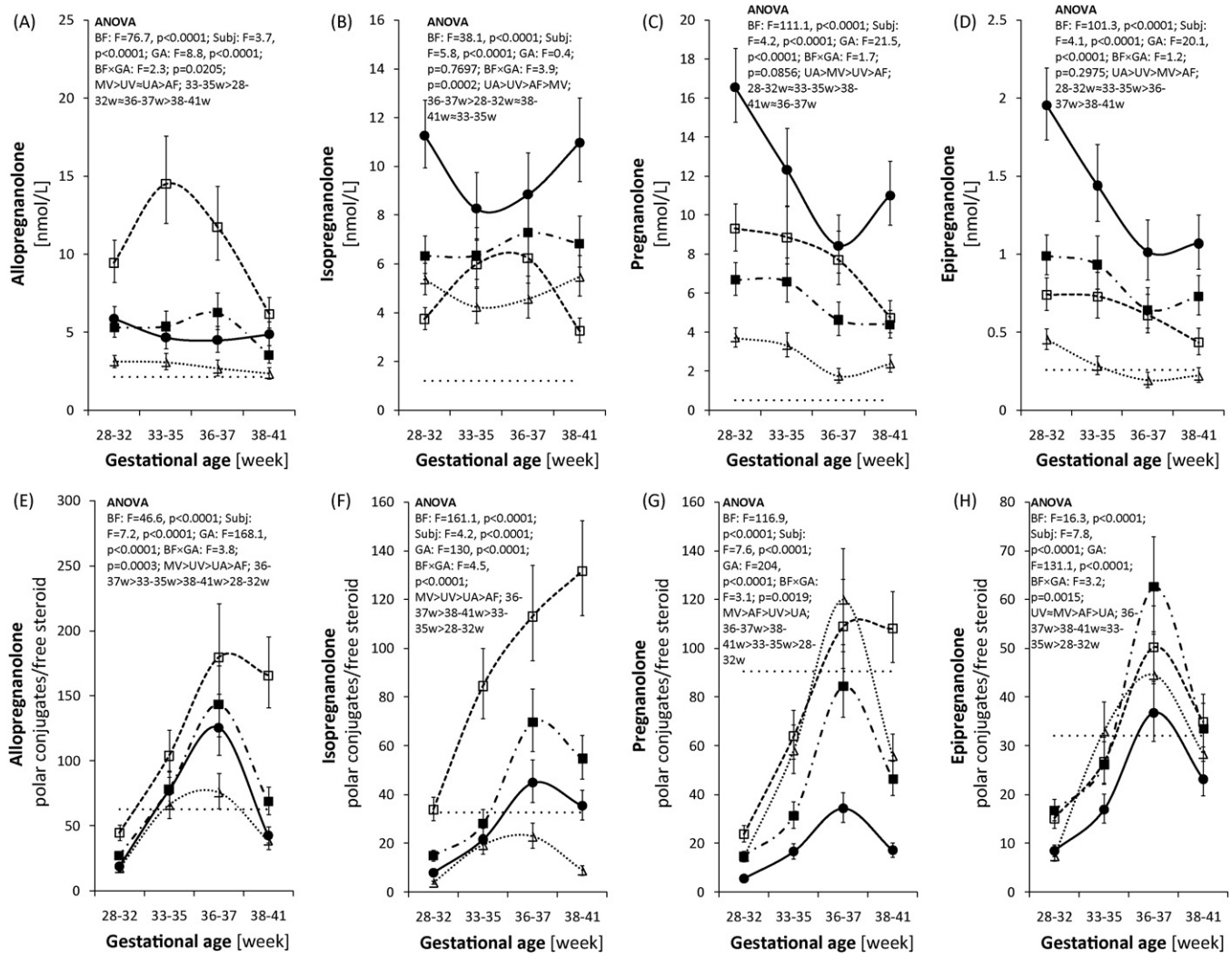

Fig. 5. Profiles of unconjugated pregnanolone isomers and ratios of conjugated pregnanolone isomers to corresponding unconjugated steroids in the plasma from the umbilical artery (UA), umbilical vein (UV) and maternal cubital vein (MV) and in amniotic fluid (AF) in preterm and normal labor. The drawings and symbols are the same as for Fig. 2. These so far unpublished data were obtained in the frame of our recently published studies [2,3].

tant role in protecting peripheral tissues from possible excessive estrogenic effects [58].

Besides placenta, also the liver shows strong expression of STS [112]. Although Warren and French [113] reported about four times less activity for DHEAS hydrolysis in human liver when compared with placenta, Selcer et al. reported comparable STS immunoreactivity in these tissues [112]. In contrast to placenta where the arylsulfatase K (ARSK) is not expressed, this enzyme might contribute to the hydrolysis of steroid sulfates in the liver [114].

3.5. Inactive steroid catabolites and prediction of term

Taking into account the simple availability of maternal blood in contrast to the fetal blood and amniotic fluid, the GA-predicting steroids in maternal plasma are of greatest interest. The inactive catabolites of sulfated Δ^5 steroids produced by the FZ or placental estrogens frequently exhibit even better predictivity for an estimation of GA that the parent steroids. These catabolites appear to be the end products of the steroid metabolism, the biosynthesis of which is readily catalyzed by the liver enzymes. For instance, the excellent predictivity was recorded for conjugated 16α-hydroxy-metabolites of Δ^5 steroids and estrogens (Fig. 6, our so far unpublished data), polar conjugates of 5α/β-reduced C-19 steroids (Fig. 7A–C, our so far unpublished data) and some 5-androstene-3β,7α/β,17β/oxo-steroids (Fig. 7D–F, our so far unpublished data) [3]. Moreover, an acceleration of 16α- and possibly also 7β-hydroxylation was reported with approaching term [61,75,76].

4. Transport of steroid sulfates into the placenta

The transport of steroid sulfates from the fetal circulation into the placental cells (where they are further metabolized) appears to be mediated by an organic anion transporter OAT-4, which is localized in the cytotrophoblast membranes and at the basal surface of the syncytiotrophoblast [115] (Fig. 8, our so far unpublished data). The data indicates the transport of steroid conjugates between the fetal and maternal compartment without preceding hydrolysis.

5. Steroid metabolism in placenta

Sex hormones produced by the placenta play a key role in the endocrine control of pregnancy and parturition. Placental CRH stimulates the production of estradiol in a time- and dose-dependent manner and also the mRNA levels of the key enzymes for estrogen synthesis such as CYP19A1, type 1 17β-HSD (HSD17B1)

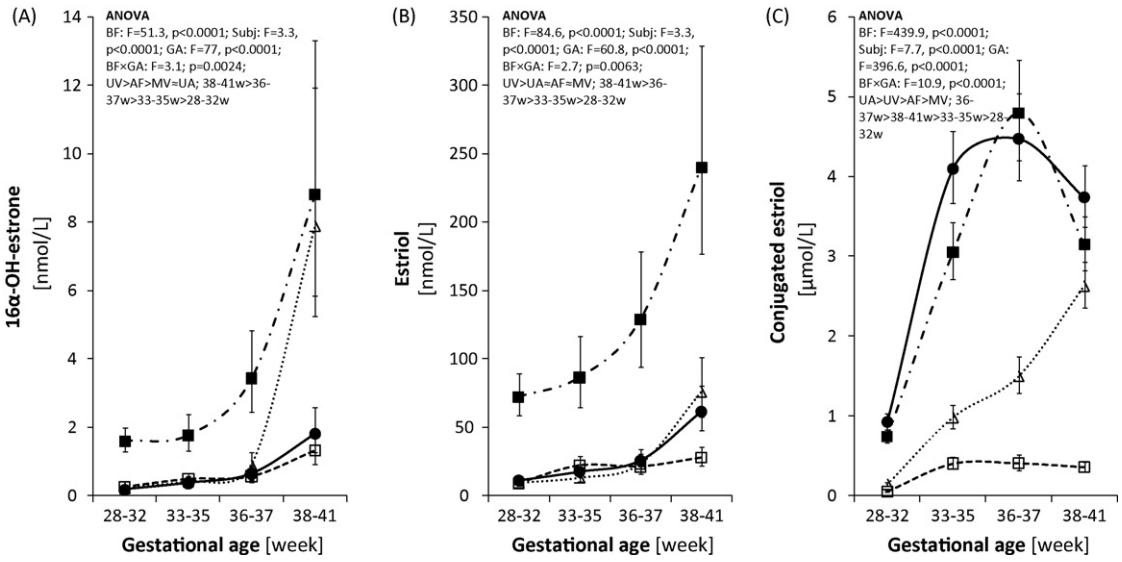

Fig. 6. Profiles of 16α-hydroxy-estrogens in the plasma from the umbilical artery (UA), umbilical vein (UV) and maternal cubital vein (MV) and in amniotic fluid (AF) in preterm and normal labor. The drawings and symbols are the same as for Fig. 2. These so far unpublished data were obtained in the frame of our recently published studies [2,3].

[116] as well as enzymes involved in progesterone synthesis like type 1 3β-HSD (HSD3B1) and CYP11A1 [117].

5.1. Cholesterol desmolase in placenta

In contrast to other tissues producing cholesterol, placenta lacks short-term modulation of steroid synthesis. In this tissue, the electron supply to CYP11A1 limits the conversion rate permitting pregnenolone synthesis to proceed at only 16% maximum velocity. Thus, the mitochondria have a near-saturating cholesterol concentration for CYP11A1, likely provided by the StAR-like protein MLN64. Cholesterol translocation to the CYP11A1 is not critical for placental progesterone synthesis and the subsequent pregnenolone conversion to progesterone [118].

5.2. Steroid sulfatases and placental production of sex hormones

The principal metabolic step preceding further placental metabolism of sulfated Δ^5 steroids originating in FZ is their desulfation being provided by the placental STS, which is localized in the endoplasmic reticulum [119]. The placental STS expression in pregnancy explicitly outweighs the production in other tissues [58]. STS allows access of DHEA to the HSD3B1 and CYP19A1 within the syncytiotrophoblast layer and conversion to estrogens [120]. Placental STS is independent of substrate concentration [121] and of GA [122–124].

5.3. 3β-Hydroxysteroid dehydrogenase activity

HSD3B1 is necessary for the placental synthesis of progesterone and C-19 3-oxo-4-ene steroids. The latter substances are further metabolized to estrogens [60,125]. HSD3B1 placental activity is predominantly located in the syncytiotrophoblast and intermediate trophoblast cells [126,127]. The specific activities of HSD3Bs for C-21 steroids in mitochondrial and microsomal preparations from human term placenta are about two times higher than for the C-19 steroids [128]. Like in the case of sulfatase activity, placental HSD3B1 activities are constant throughout the human gestation [129,122,123] and around parturition [124,130]. Progesterone and DHEAS may cause marked HSD3Bs inhibition in physiological conditions [121,131].

5.4. Estrogen formation

Placenta is the primary site of estrogen formation in pregnancy. CYP19A1 catalyzing the last steps of estrogen biosynthesis from Δ^4 C-19 steroids is abundantly expressed in syncytiotrophoblast [132]. Estrogens regulate their own synthesis by the product inhibition. The substrate inhibition is more apparent for 16-deoxy-estrogens than for their 16α-hydroxy-metabolites [133]. 16-Deoxy- and 16-hydroxy-C-19 substrates bind at separate, but interactive sites and each substrate on binding inhibits the aromatization of the other [134,135].

CYP19A1 activity strongly depends on GA. The increase in estradiol levels in maternal blood from the 2nd to the 3rd trimester is greater than that of the placental weight and there is significantly higher placental CYP19A1 activity in the 3rd trimester than in the 2nd trimester [122]. The aforementioned results as well as our recent data [3] indicate high predictivity of parturition onset for some estrogens (Fig. 9, our so far unpublished data).

5.5. 16α-Hydroxylation

Although the CYP3A7 is primarily expressed in the fetal liver, its activity was also found in the placenta. The amounts of placental and endometrial CYP3A7 mRNA and protein substantially increase from the first to the second trimester of pregnancy [73].

5.6. 5α/β-Reductases

The pioneer studies on placental 5α-reductase [129] reported *in vitro* synthesis of 5α-reduced pregnanes [3H]5α-pregnane-3,20-dione and [3H]3β-hydroxy-5α-pregnan-20-one from [3H]progesterone by placental tissue. 5α-Reduced steroids, including allopregnanolone, suppress neuronal activity and may have neuroprotective effects in the fetus. Placental expression of both isoenzymes increased with advancing gestation. Placental 5α-reductases may provide precursors for allopregnanolone synthesis in fetal brain [136].

AKR1D1 is primarily expressed in the liver but its activity was also detected in other tissues including placenta [30]. The progesterone metabolite 5β-dihydroprogesterone (5β-DHP) is a potent tocolytic. Acute *in vitro* treatment with 5β-DHP causes rapid uter-

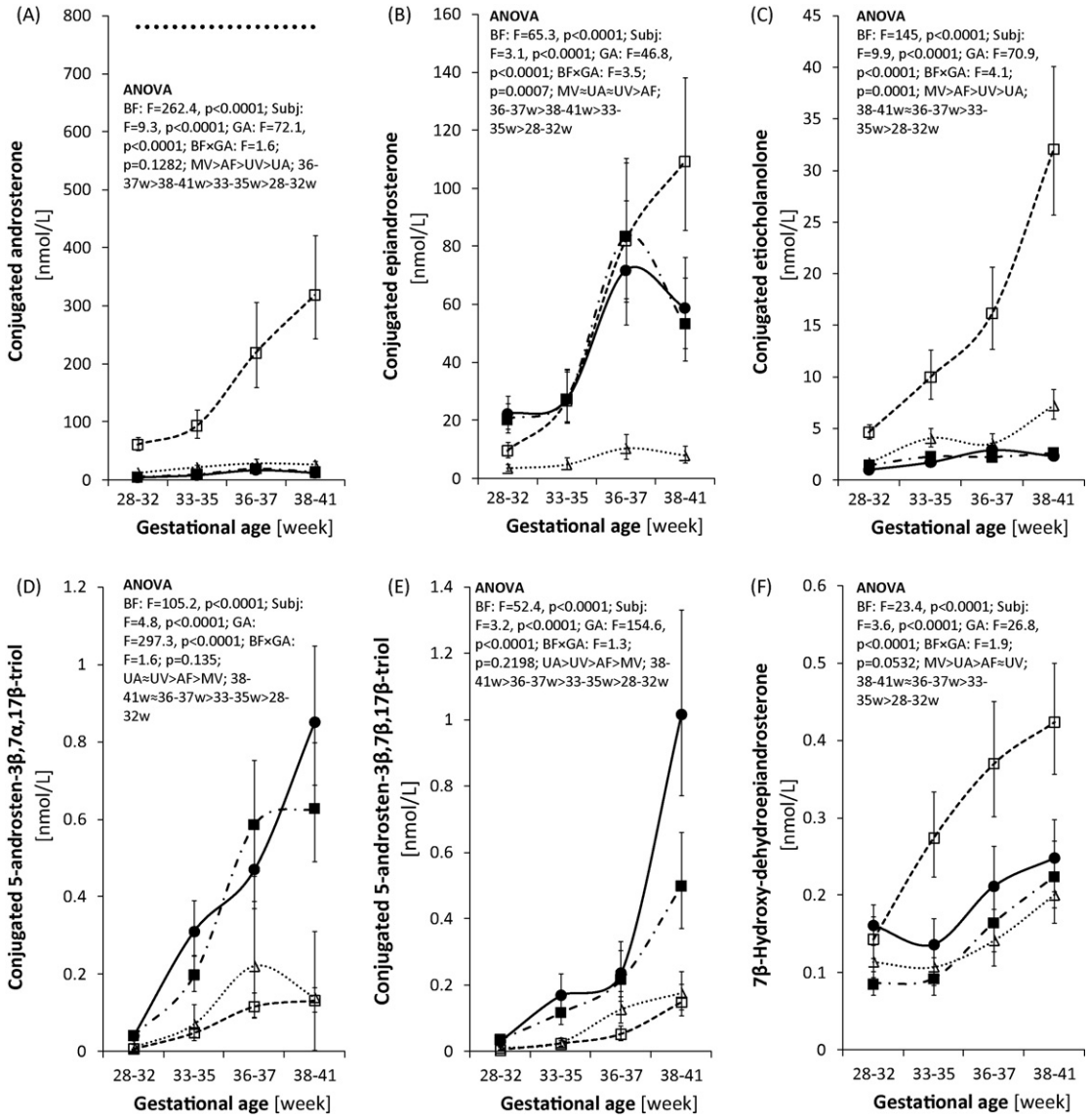

Fig. 7. Profiles of three conjugated 3α/β-hydroxy-5α/β-androstane-17-ones and some 5-androstene-3β, 7α/β,17β/17-oxo-steroids in the plasma from the umbilical artery (UA), umbilical vein (UV) and maternal cubital vein (MV) and in amniotic fluid (AF) in preterm and normal labor. The drawings and symbols are the same as for Fig. 2. These so far unpublished data were obtained in the frame of our recently published studies [2,3].

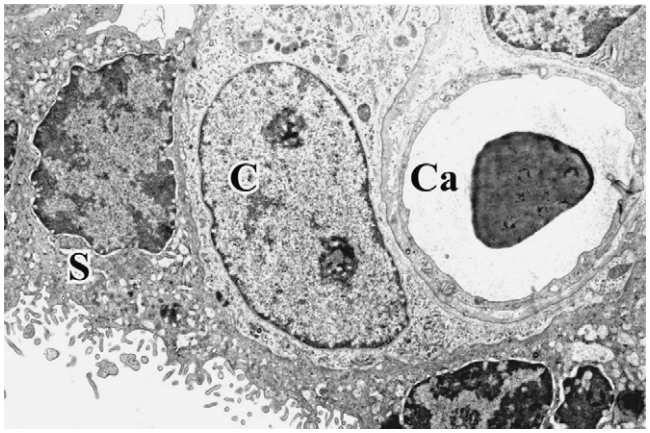

Fig. 8. Transmission electron micrograph of the human placenta; S, syncytiotrophoblast; C, cytotrophoblast; Ca, fetal villous capillary; bar, 2 μm (our so far unpublished data).

ine relaxation that is not mediated by pregnane X-type receptors (PXR) but the 5β-reduced metabolites of progesterone may also act chronically in pregnancy through a PXR-mediated mechanism [29]. In the placenta and myometrium, relative expression of AKR1D1 decreases in association with labor by about 2-fold and 10-fold, respectively [30]. In contrast to the turnover of progesterone to 5α-DHP reflecting 5α-reductase activity which remains stable (Fig. 4C, our so far unpublished data), the conversion of progesterone to 5β-DHP reflecting 5β-reductase activity decreases later in pregnancy [13,26,30] (Fig. 4D, our so far unpublished data). This data is consistent with a possible role for 5β-DHP in the onset of spontaneous human parturition. The placental expression of 5β-reductase mRNA is about two orders of magnitude higher than in myometrium and about three orders of magnitude higher than in chorion and amnion [30].

5.7. Steroid sulfotransferase

SULT2B1 catalyzing sulfation of DHEA but not estradiol is present in syncytiotrophoblast [107,108,137,138] while SULT1E1

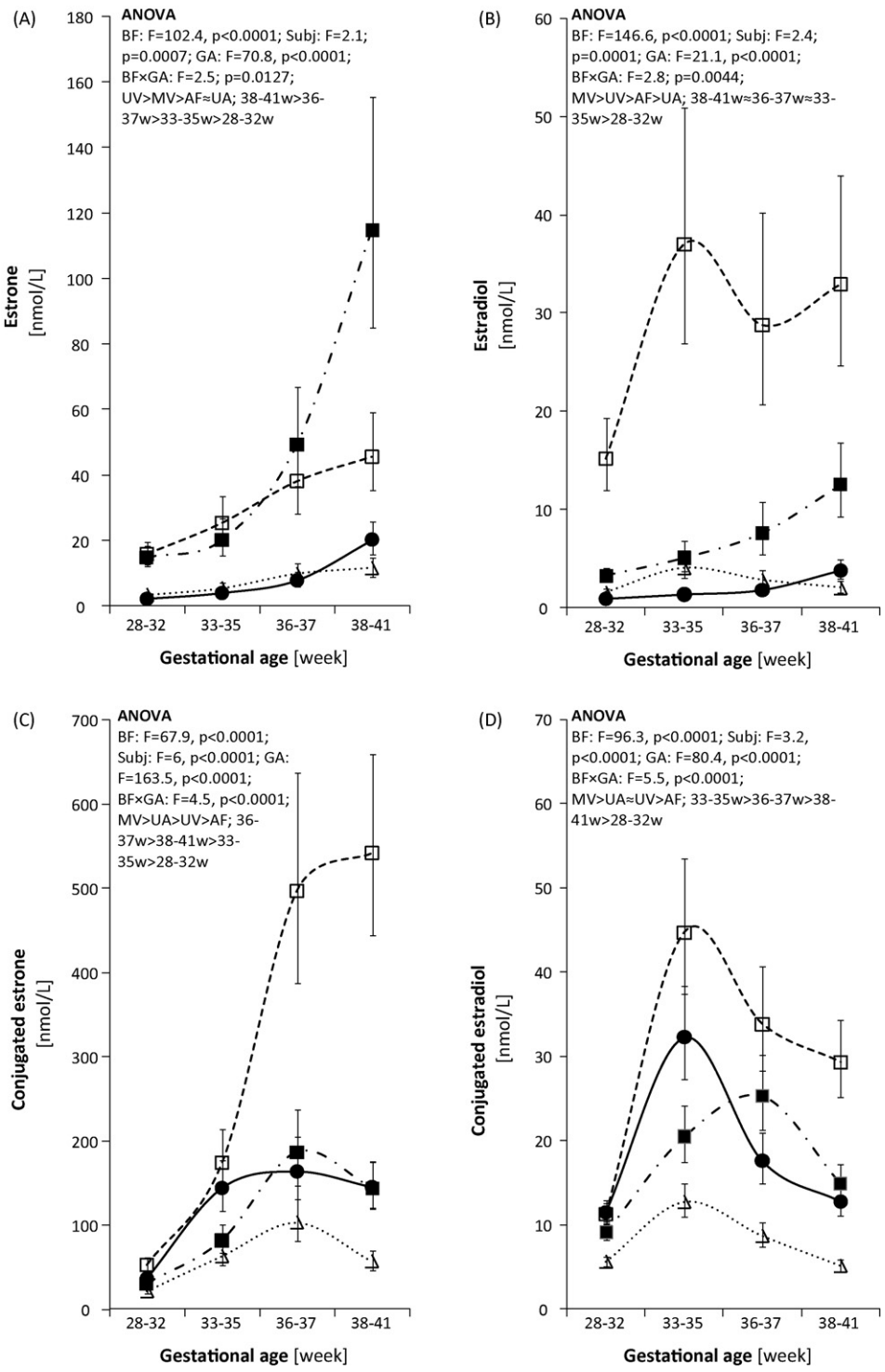

Fig. 9. Profiles of free and conjugated estrogens in the plasma from the umbilical artery (UA), umbilical vein (UV) and maternal cubital vein (MV) and in amniotic fluid (AF) in preterm and normal labor. The drawings and symbols are the same as for Fig. 2. These so far unpublished data were obtained in the frame of our two recently published studies [2,3]. These so far unpublished data were obtained in the frame of our recently published studies [2,3].

and SULT2A1 show negligible functional activity in placental tissues [139], which means that estrogens are sulfated in an extra placental way, most probably in the liver.

5.8. Reversible C-3, C-11, C-17 and C-20 oxidoreductive inter-conversions in placenta

Placenta expresses various dehydrogenases belonging to SDRs and AKRs. From the SDRs, the cytoplasmic HSD17B1 is highly expressed in syncytiotrophoblast [42]. Besides catalyzing the conversion of estrone and progesterone to estradiol and Prog20α, respectively, HSD17B1 may also catalyze the formation of 5-androstene-3β,17β-diol from DHEA [140,141]. Syncytiotrophoblast, coming directly into contact with maternal blood, converts estrone to estradiol. In contrast to type HSD17B1 mRNA, type HSD17B2 mRNA is not detectable in cell cultures of human cytotrophoblast or syncytiotrophoblast [142]. Besides HSD17B1, the AKR1 member C3 enzyme (AKR1C3), HSD17B7 and HSD17B12

may also catalyze progesterone deactivation to Prog20α and conversion of inactive estrone to bioactive estradiol [68,88,132,141].

AKR1C3 is pluripotent widely distributed enzyme catalyzing the conversion of aldehydes and ketones to alcohols [143,144]. AKR1C3 functions as a bi-directional 3α-, 17β- and 20α-HSD and can interconvert active androgens, estrogens and progestins with their cognate inactive metabolites, however, like other AKR1Cs *in vivo*, AKR1C3 preferentially works as a reductase [88,91,143].

Regarding 3α-pregnanolone isomers positively modulating $GABA_A$-r, the oxidoreductive conversion of 20-oxo- to 20α-hydroxy-group or a modification of the C17,20 side chain results in a selective (subtype dependent) reduction of positive allosteric modulation of $GABA_A$-r (about 6-fold) [145]. In addition, the reversible oxidoreductive interconversion of 3α-hydroxy/3-oxo/3β-hydroxy-5α/β-reduced pregnane and androstane steroids may influence the ratio of neuroinhibiting 3α-hydroxy-5α/β-reduced metabolites, which are allosteric positive modulators of $GABA_A$-r, to the corresponding 3-oxo-metabolites and 3β-hydroxy-metabolites. The latter ones are biologically inactive but compete with the 3α-hydroxy-isomers for the active sites on the receptors [146].

In contrast to the aforementioned enzymes, the HSD17B2 prefers the oxidative direction catalyzing the progesterone biosynthesis from inactive Prog20α as well as the conversion of bioactive estradiol to biologically inactive estrone [42]. The site of expression of HSD17B2 was identified in two studies, either in endothelial cells of fetal capillaries and some stem villous vessels [42] or in endothelial cells of villous arteries and arterioles [147]. Moghrabi et al. suggested a protective role of the HSD17B2 from the excess of bioactive estrogens and androgens in the fetus [42]. Besides HSD17B2, the type 14 17β-HSD (HSD17B14) a member of SDRs may also convert estradiol to estrone and 5-androstene-3β,17β-diol to DHEA [147].

The metabolism of placental sex steroids in the reductive direction increases as pregnancy advances and significantly rises during human parturition [129,148]. This phenomenon may be of an importance in the mechanism of initiation and continuation of labor and might indicate a mechanism of progesterone withdrawal in association with the onset of human parturition.

HSD11B1 expression is abundant in syncytiotrophoblast microvillus membranes juxta the maternal circulation whereas HSD11B2 expression is extensive throughout the remainder of the syncytiotrophoblast, including the basal cell membrane and epithelial basal lamina [149]. HSD11B1 expression is constant, but the expression of HSD11B2 in the placenta increases significantly with GA. The adaptation of HSD11B2 activity prevents the increasing maternal cortisol concentrations from transplacental passage [150].

Distribution of placental oxidoreductases and sources of progesterone, estrogens and neuroactive steroids in pregnancy.

As indicated by growing progesterone levels in UV (Fig. 10, our so far unpublished data), placental production of progesterone probably increases shortly before termination of pregnancy but its levels in UA, maternal cubital vein (MV), and amniotic fluid (AF) remain constant. This means that there should be concurrently increasing progesterone catabolism in this period.

Paradoxically, although progesterone is the most important steroid in human pregnancy there are a lot of peculiarities and contradictions regarding its biosynthesis. As already mentioned, the FZ is analogous to adult *zona reticularis*. However, while both FZ and *zona reticularis* produce large amounts of DHEAS, the extensive production of PregS is specific for FZ. This substantial dissimilarity between FZ and *zona reticularis* remains unexplained. Although, DHEAS from the FZ is generally accepted as the substrate for placental estrogen synthesis, the physiological role of PregS in human pregnancy is unknown.

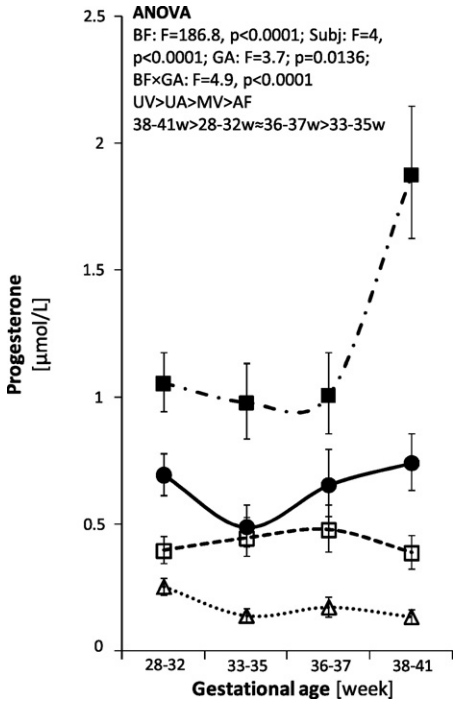

Fig. 10. Profile of progesterone in the plasma from the umbilical artery (UA), umbilical vein (UV) and maternal cubital vein (MV) and in amniotic fluid (AF) in preterm and normal labor. The drawings and symbols are the same as for Fig. 2. These so far unpublished data were obtained in the frame of our recently published studies [2,3].

On the other hand, the maternal LDL cholesterol is considered to be a single substrate for placental progesterone synthesis [7,45], although the conversion of cholesterol/sulfate to pregnenolone/sulfate is the rate limiting step but not the cholesterol transport to active sites like in extra placental tissues. It is generally accepted that the activities of STS and HSD3B1 are enormous in comparison with other human tissues and being independent of GA are capable to readily convert DHEAS to estrogens. Inconsistently, the fate of PregS was not considered although its concentrations in late pregnancy are at least the same as DHEAS levels. Whereas DHEAS easily penetrates to the active sites in placenta being desulfated and converted to androstenedione and testosterone, there is no reason for PregS to act differently. Neither STS nor HSD3B1 activities are rate limiting for placental progesterone synthesis [118]. Therefore, it may be more expediential to utilize fetal PregS instead of the necessity to synthesize total maternal progesterone *de novo* from maternal LDL cholesterol.

The progesterone is vital for pregnancy sustaining and so there may be independent sources for its production. Whereas the conversion of maternal LDL cholesterol may be the first of them, there is no reason for placenta to reject processing of pregnenolone sulfate in the same way as DHEAS, obviously, except for the final step, i.e. estrogen synthesis. The former source provides stability of progesterone production in the cases where the steroid production in the FZ fails, however, the latter may substantially contribute to the progesterone production. As our data indicates, the rise in production of Δ^5 steroids with approaching labor is linked to rise of progesterone levels in UV but not in other body fluids. This data indicates that primarily placental and perhaps also the liver oxidoreductases may readily convert progesterone to its metabolite Prog20α and *vice versa* and that the different location of the reductase- or oxidoreductase-preferring isoforms in placental tissues may be decisive for the reductive or oxidative status of steroid metabolome in mother and fetus. Not only the progesterone levels,

Table 1
Correlations (after power transformation to Gaussian distribution and constant variance) between progesterone (Prog), 20α-dihydroprogesterone (Prog20α), and 20α-dihydroprogesterone polar conjugates (Prog20αC) in umbilical venous blood (UV) and maternal venous blood (MV); Pearson's and partial correlations (with adjustment of all variables in the correlation matrix to constant except the pair under investigation) are above and below the diagonal, respectively.

		UV			MV			
		Prog	Prog20α	Prog20αC	Prog	Prog20α	Prog20αC	
UV	Prog		0.277 46 0.063	0.196 46 0.192	0.083 46 0.582	0.103 46 0.497	0.188 46 0.210	PEARSON'S CORRELATIONS
	Prog20α	0.241 46 0.124		**0.569** **46** **0.000**	**0.326** **46** **0.027**	**0.632** **46** **0.000**	**0.527** **46** **0.000**	
	Prog20αC	0.030 46 0.852	0.254 46 0.105		0.202 46 0.178	**0.515** **46** **0.000**	**0.747** **46** **0.000**	
MV	Prog	0.090 46 0.569	-0.124 46 0.433	-0.135 46 0.395		**0.680** **46** **0.000**	**0.310** **46** **0.036**	
	Prog20α	-0.156 46 0.323	**0.432** **46** **0.004**	0.071 46 0.655	**0.647** **46** **0.000**		**0.602** **46** **0.000**	
	Prog20αC	0.072 46 0.651	-0.016 46 0.922	**0.597** **46** **0.000**	-0.032 46 0.842	0.283 46 0.070		
		PARTIAL CORRELATIONS						

but also the concentration of estrogens, NAS and other substances which influence pregnancy sustaining like 5α/β reduced pregnane and androstane metabolites in fetal and maternal circulations are in all probability controlled by the distribution of placental oxidoreductases.

The reason why the placental production was considered to be independent of the fetal PregS might be the absence of correlations between maternal and fetal progesterone although the levels of estradiol (synthesized from the fetal DHEAS) also do not correlate between mother and fetus. However, as reported in our recent study [2], there are significant partial correlations for both free and conjugated Prog20α between UV and MV and the correlation between Prog20α and progesterone in MV (Table 1). In addition, there are also significant partial correlations between estrone polar conjugates in UV and unconjugated estradiol in MV and a correlation between estrone and estradiol in MV (Table 2) (our unpublished data).

Assuming that the distribution of placental oxidoreductase isoforms controls the reductive and oxidative status of steroid inter-conversions in maternal and fetal compartment, respectively, the difference between oxidative fetal and reductive maternal steroid metabolomic status should be the most apparent when comparing blood from UV, containing placental steroids before their further metabolism in other fetal tissues (mainly liver), and MV. In accordance with the aforementioned assumption, the blood from UV contains higher proportions of 20-oxo-steroids like progesterone, 17-oxo-steroids (e.g. estrone and DHEA), 3-oxo-steroids like 5α/β-DHP and 3β-hydroxysteroids (isopregnanolone and epipregnanolone), while maternal venous blood contains higher proportions of 20α-hydroxysteroids like 20α-dihydroprogesterone, 17β-hydroxysteroids such as estradiol and

Table 2
Correlations (after power transformation to Gaussian distribution and constant variance) between estrone (E1), estrone polar conjugates (E1C), estradiol (E2), and estradiol polar conjugates (E2C), in umbilical venous blood (UV) and maternal venous blood (MV); Pearson's and partial correlations (with adjustment of all variables in the correlation matrix to constant except the pair under investigation) are above and below the diagonal, respectively.

		UV				MV				
		E1	E1C	E2	E2C	E1	E1C	E2	E2C	
UV	E1		**0.540** **45** **0.000**	**0.702** **45** **0.000**	**0.337** **46** **0.022**	**0.427** **47** **0.003**	**0.615** **46** **0.000**	**0.355** **47** **0.014**	**0.366** **47** **0.011**	PEARSON'S CORRELATIONS
	E1C	-0.015 45 0.930		**0.596** **46** **0.000**	**0.557** **48** **0.000**	**0.335** **48** **0.020**	**0.853** **48** **0.000**	**0.396** **48** **0.005**	**0.629** **48** **0.000**	
	E2	**0.623** **45** **0.000**	0.109 46 0.503		**0.691** **47** **0.000**	**0.343** **47** **0.018**	**0.610** **46** **0.000**	0.278 47 0.059	**0.421** **47** **0.003**	
	E2C	**-0.395** **46** **0.012**	0.024 48 0.881	**0.698** **47** **0.000**		0.226 49 0.119	**0.507** **48** **0.000**	**0.430** **49** **0.002**	**0.683** **49** **0.000**	
MV	E1	0.001 47 0.998	-0.189 48 0.231	0.185 47 0.248	-0.207 49 0.182		**0.458** **49** **0.001**	**0.614** **50** **0.000**	**0.406** **50** **0.004**	
	E1C	0.201 46 0.215	**0.691** **48** **0.000**	0.042 46 0.796	-0.035 48 0.824	0.279 49 0.070		**0.397** **49** **0.005**	**0.612** **49** **0.000**	
	E2	0.194 47 0.224	0.075 48 0.636	-0.195 47 0.223	0.152 49 0.332	**0.526** **50** **0.000**	-0.180 49 0.249		**0.658** **50** **0.000**	
	E2C	0.072 47 0.656	0.149 48 0.347	-0.280 47 0.076	**0.503** **49** **0.001**	0.002 50 0.988	0.191 49 0.220	**0.399** **50** **0.007**		
		PARTIAL CORRELATIONS								

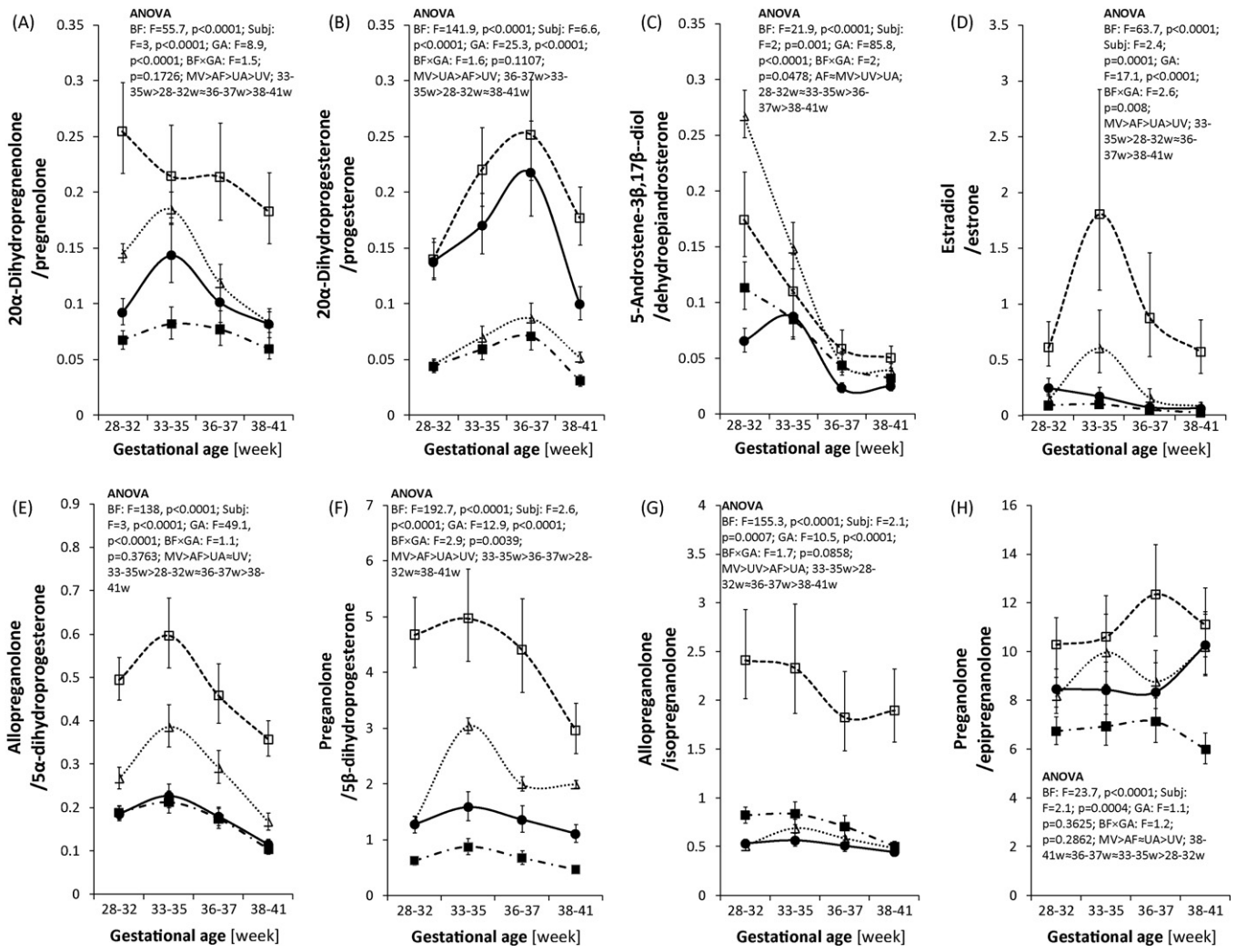

Fig. 11. Profiles of the ratios of steroids in reduced forms to the corresponding oxidized forms in the plasma from the umbilical artery (UA), umbilical vein (UV) and maternal cubital vein (MV) and in amniotic fluid (AF) in preterm and normal labor. The drawings and symbols are the same as for Fig. 2. These so far unpublished data were obtained in the frame of our recently published studies [2,3].

androstenediol and 3α-hydroxysteroids like neuroinhibiting allopregnanolone and pregnanolone (Fig. 11, our so far unpublished data). Furthermore, the levels of conjugated 3α-hydroxy-5α/β-reduced C-19 steroids in MV are pronouncedly higher (Fig. 7A–C, our so far unpublished data) than in the fetal circulation and amniotic fluid, while the 3β-isomer conjugated epiandrosterone do not significantly differ between mother and fetus (Fig. 7B, our so far unpublished data).

Besides the neuroinhibiting effects in the CNS (which are probably counterbalanced by changed phosphorylation status of the $GABA_A$-r [10]) the higher levels of the 3α-hydroxysteroids in MV might be useful for pregnancy sustaining by reducing myometrial activity via the voltage-gated K^+ channels [105].

Gilbert Evans and colleagues reported 3α-hydroxysteroid oxidoreductase-mediated turnover of 5α- and 5β-DHP to their metabolites allopregnanolone and pregnanolone. In the maternal circulation, between the 28th and 38th week of gestation, a decrease of allopregnanolone and increase of pregnanolone occurred [26]. On the contrary, our data showed a consistent decrease in both allopregnanolone/5α-DHP and pregnanolone/5β-DHP ratios between the 28th and 41st week of gestation (Fig. 11E and F, our so far unpublished data). In addition, we have recorded a slight but significant decrease even in the allopregnanolone/isopregnanolone ratio (Fig. 11G, our so far unpublished data). Considering the enzyme distribution in placenta, these results indicate increasing activity of placental and perhaps also the liver HSD17B7 in late pregnancy. The data also points to decreasing synthesis of neuroinhibiting GABA-ergic steroids with advancing gestation.

6. Conclusions

The data available in the literature including our recent findings and new unpublished data indicate increasing progesterone synthesis that is accompanied by increasing catabolism with approaching parturition. The data also confirms declining production of pregnancy sustaining 5β-pregnane steroids. These substances provide uterine quiescence in late pregnancy. There is also an increasing sulfation of neuroinhibiting and pregnancy sustaining steroids with approaching term. In contrast to the established concept considering LDL cholesterol as the primary substrate for progesterone synthesis in pregnancy, our data demonstrates the functioning of alternative mechanism for progesterone synthesis, which is based on the utilization of fetal pregnenolone sulfate for progesterone production in placenta. Close relationships were found between localization of placental oxidoreductases and consistently higher levels of sex hormones, neuroactive steroids and their metabolites in the oxidized form

in the fetus and in the reduced form in the maternal compartment.

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