

Reduced progesterone metabolites in human late pregnancy

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Short title

Neuroactive steroids in late pregnancy

Summary

In this review, we focused on the intersection between steroid metabolomics, obstetrics and steroid neurophysiology to give a comprehensive insight into the role of sex hormones and neuroactive steroids (NAS) in the mechanism controlling pregnancy sustaining. The data in the literature including our studies show that there is a complex mechanism providing synthesis of either pregnancy sustaining or parturition provoking steroids. This mechanism includes the boosting placental synthesis of CRH with approaching parturition inducing the excessive synthesis of 3β -hydroxy-5-ene steroid sulfates serving primarily as precursors for placental synthesis of progestogens, estrogens and NAS. The distribution and changing activities of placental oxidoreductases are responsible for the activation or inactivation of the aforementioned steroids, which is compartment-specific (maternal and fetal compartments) and dependent on gestational age, with a tendency to shift the production from the pregnancy-sustaining steroids to the parturition provoking ones with an increasing gestational age. The fetal and maternal livers catabolize part of the bioactive steroids and also convert some precursors to bioactive steroids. Besides the progesterone, a variety of its $5\alpha/\beta$ -reduced metabolites may significantly influence the maintenance of human pregnancy, provide protection against excitotoxicity following acute hypoxic stress, and might also affect the pain perception in mother and fetus.

Keywords: neuroactive steroids, pregnancy, plasma, metabolome, GC-MS

1 Introduction

Although the effects of neuroactive and neuroprotective $5\alpha/\beta$ -reduced progesterone metabolites were extensively studied, the physiological relevance of these substances remains frequently uncertain due to the lack of metabolomic data. We attempted to elucidate the role of sex hormones and neuroactive steroids (NAS) in the mechanism controlling the pregnancy maintenance and parturition onset.

2 Biosynthesis of neuroactive steroids in human pregnancy

2.1 Steroid metabolism in fetal and maternal adrenal

2.1.1 Placental CRH regulate the steroid biosynthesis in pregnancy

The machinery regulating production of pregnancy steroids is based on the excessive placental production of corticoliberin (CRH) (Figure 1) (Goland *et al.* 1986, Rainey *et al.* 2004, Smith *et al.* 2009). Pregnant women after luteo-placental shift produce CRH primarily in the 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.

The rising CRH levels in the last four weeks of pregnancy stimulate the synthesis of conjugated Δ^5 steroids in the fetal adrenal (Sirianni *et al.* 2005, Smith *et al.* 1998). CRH is as effective as ACTH at stimulating sulfated dehydroepiandrosterone (DHEAS) production but is 70% less potent than ACTH at stimulating cortisol production. The excessive production of placental CRH is specific for primates and the boosting CRH production near term is specific only for humans and great apes (Power and Schulkin 2006). The sulfated Δ^5 steroids, originating in the fetal zone of the fetal adrenal (FZ), represent the largest fraction of steroids in pregnancy (Ingelman-Sundberg *et al.* 1975, Lacroix *et al.* 1997, Leeder *et al.* 2005, Moghrabi *et al.* 1997). The FZ is similar to the adult *zona reticularis* but unlike the adult *zona reticularis*, the FZ produces excessive amounts of sulfated C-21 Δ^5 steroids, including sulfates of pregnenolone and 17-hydroxypregnenolone (Hill *et al.* 2010a, Hill *et al.* 2010b, Rainey *et al.* 2004). These substances serve as precursors for the placental production of estradiol (Sirianni *et al.* 2005, Smith *et al.* 1998) and progesterone (Hill *et al.* 2010b, Jaffe and Ledger 1966, Komatsuzaki *et al.* 1987, Walsh 1988).

2.2 Steroid metabolism in fetal and maternal liver

2.2.1 C-3, C-17 and C-20 oxidoreductive conversions

The enzymes, 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). Human liver contains various isoforms of AKRs (AKR1C1, AKR1C2, AKR1C3, and AKR1C4) with 20 α -, 17 β -, 3 α - or 3 β -hydroxysteroid dehydrogenase-like activity (Jin *et al.* 2009, Penning *et al.* 2001, Shiraishi *et al.* 1998). AKRs activities could control occupancy of the androgen- and GABA_A-receptors (Penning 1999) via reduction of oxo-groups mostly in the steroid C3 and C17 positions, respectively. *In vivo*, all AKR1Cs preferentially work as reductases (Steckelbroeck *et al.* 2004) and are capable to reduce estrone, progesterone, and 3-oxo-pregnane/androstane steroids to estradiol, 20 α -dihydroprogesterone, and GABAergic 3 α -hydroxy-5 α / β -pregnane/androstane steroids, respectively. On the other hand, AKR1Cs may decrease the concentrations of GABAergic steroids by inactivating allopregnanolone and eliminating the precursors like progesterone from the synthetic pathways via reduction of the 20-oxo-steroid group (Penning *et al.* 2000, Usami *et al.* 2002). The AKR1C2 preferring 3 α -reduction over the 3 β -reduction may catalyze 3 α -, 17 β -, and 20 α -hydroxysteroid dehydrogenase (HSD) reactions (HSD) (Jin *et al.* 2009, Jin *et al.* 2001, Penning *et al.* 2000, Usami *et al.* 2002). AKR1C3 catalyzes the reduction of 5 α -dihydrotestosterone (5 α -DHT), androstenedione, estrone and progesterone to produce 5 α -androstane-3 α ,17 β -diol, testosterone, estradiol and 20 α -dihydroprogesterone, respectively (Penning *et al.* 2001). AKR1C4, the expression of which is specific for the liver (Nishizawa *et al.* 2000, Penning *et al.* 2000, Penning *et al.* 2004), catalyzes the transformation of the 5 α -DHT into 5 α -androstane-3 α ,17 β -diol. Liver specific AKR1C4 shows superior catalytic efficiency exceeding 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 (Jin *et al.* 2009). From the SDRs, the type 7 17 β -HSD (HSD17B7), preferring the reduction of the oxo-groups in 20-, 17- and 3-positions to the corresponding 20 α -hydroxy-, 17 β -hydroxy-, and 3 α -hydroxy-counterparts, is also significantly expressed in the liver (Krazeisen *et al.* 1999, Torn *et al.* 2003) as well as type 12 17 β -HSD (HSD17B12) catalyzing the transformation of estrone into the estradiol (Sakurai *et al.* 2006).

On the other hand, type 2 17 β -HSD (HSD17B2), type 10 17 β -HSD (HSD17B10) and type 11 17 β -HSD (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 20 α - and 17 β - counterparts (Moghrabi *et al.*

1997). Type 6 17 β -HSD (HSD17B6) prefers oxidoreductase and 3(α - \rightarrow β)-hydroxysteroid epimerase activities and acts on both C-19 and C-21 3 α -hydroxysteroids (Huang and Luu-The 2000). HSD17B10 being abundantly expressed in the liver, is capable of catalyzing the oxidation of steroid modulators of GABA_A-r (He *et al.* 2001). HSD17B10 catalyzes the oxidation of 5 α -androstane-3 α ,17 β -diol to 5 α -DHT (He *et al.* 2003) and conversion of 3 α -hydroxy-5 α -pregnane steroids to the corresponding inactive 3-oxo steroids. The catalysis of HSD17B10 is essential for maintaining normal functions of GABAergic neurons (Shafiqat *et al.* 2003). Finally, the HSD17B11 can convert 5 α -androstane-3 α ,17 β -diol to androsterone (Brereton *et al.* 2001, Chai *et al.* 2003).

2.2.2 5 α / β -Reductases

Steroid 5 α / β reduction is one of the key steps for the biosynthesis of various neuroactive and neuroprotective substances. The liver has high activity of 5 α -reductase (SRD5A) and 5 β -reductase (AKR1D1) (Charbonneau and The 2001, Meikle *et al.* 1979). Type 1 SRD5A (SRD5A1) is widely distributed in the body, with the highest levels in the liver. SRD5A1 converts testosterone into 5 α -dihydrotestosterone and progesterone or corticosterone to their 5 α -reduced counterparts. In the peripheral tissues, including the liver, SRD5A1 and reductive 3 α -HSD isoforms work consecutively eliminating the androgens, protecting against the hormone excess (Jin and Penning 2001) and producing GABAergic steroids, which are, however, extensively sulfated in the liver. Liver 5 β -reductase (AKR1D1) belonging to AKRs, efficiently catalyzes the reduction of both C-19 and C-21 3-oxo- Δ^4 steroids to the corresponding 5 β -reduced metabolites (Kochakian 1983, Okuda and Okuda 1984).

The higher levels of 5 β -reduced progesterone metabolites in the fetus than in maternal compartment, as well as the arteriovenous differences in the fetus indicate that steroid 5 α / β -reductions in the fetal liver and not in the placenta are important for production of GABAergic 5 α / β -reduced NAS in both maternal and fetal compartments (Hill *et al.* 2010a).

2.3 Steroid metabolism in placenta

2.3.1 Steroid sulfatases and placental production of sex hormones

The principal metabolic step that is indispensable for further placental metabolism of sulfated Δ^5 steroids originating in FZ is their desulfation, which is catalyzed by the placental STS. Placental STS activity is independent of substrate concentration (Watanabe *et al.* 1990) and of gestational age (GA) (Fukuda *et al.* 1986,

Ishida *et al.* 1985, Leslie *et al.* 1994). The placental STS expression in pregnancy explicitly outweighs the expression in other tissues (Miki *et al.* 2002). STS allows access of Δ^5 steroids to the type 1 3β -HSD (HSD3B1) and aromatase (CYP19A1) within the syncytiotrophoblast layer and their conversion to estrogens (Siiteri 2005) and progestogens (Hawes *et al.* 1994, Hill *et al.* 2010a, Hill *et al.* 2010b, Jaffe and Ledger 1966, Komatsuzaki *et al.* 1987, Mason *et al.* 1993, Walsh 1988).

2.3.2 3β -hydroxysteroid dehydrogenase activity

HSD3B1 placental activity is predominantly located in the syncytiotrophoblast (Mitchell and Powell 1984, Riley *et al.* 1992). Like the STS activity, the placental HSD3B1 activity is constant throughout the human gestation (Fukuda *et al.* 1986, Ishida *et al.* 1985, Milewich *et al.* 1978) and around parturition (Leslie *et al.* 1994, Riley *et al.* 1993).

2.3.3 $5\alpha/\beta$ -Reductases

Besides the liver SRD5A, also the placental SRD5A may provide precursors for allopregnanolone synthesis in fetal brain (Vu *et al.* 2009). Although AKR1D1, catalyzing the 5β -reduction is primarily expressed in the liver, its activity was also detected in placenta (Sheehan *et al.* 2005); however, placental AKR1D1 activity appears to be minor in comparison with that in the liver (Hill *et al.* 2010a, Milewich *et al.* 1979). The progesterone metabolite 5β -DHP is a potent tocolytic (Mitchell *et al.* 2005). In the placenta and myometrium, expression of AKR1D1 decreases in association with labor by about two-fold and 10-fold, respectively (Sheehan *et al.* 2005). In contrast to the turnover of progesterone to 5α -dihydroprogesterone (5α -DHP) reflecting SRD5A activity, which remains stable (Hill *et al.* 2010a), the conversion of progesterone to 5β -DHP reflecting AKR1D1 activity decreases later in pregnancy (Gilbert Evans *et al.* 2005, Hill *et al.* 2007, Sheehan *et al.* 2005).

2.3.4 Reversible C-3, C-17 and C-20 oxidoreductive inter-conversions in placenta and fetal membranes

From the SDRs, the cytoplasmic type 1 17β -HSD (HSD17B1) is highly expressed in the syncytiotrophoblast (Moghrabi *et al.* 1997). Besides catalyzing the conversion of estrone and progesterone to estradiol and 20α -dihydroprogesterone, respectively, HSD17B1 may also convert DHEA to 5-androstene- $3\beta,17\beta$ -diol (Lin *et al.* 2006, Peltoketo *et al.* 1999). Syncytiotrophoblast, coming directly into contact with maternal blood, converts

biologically inactive estrone to bioactive estradiol. In contrast to the HSD17B1 mRNA, HSD17B2 mRNA is not detectable in cell cultures of human cytotrophoblast or syncytiotrophoblast (Bonenfant *et al.* 2000b).

Besides HSD17B1, the AKR1 member C3 enzyme (AKR1C3), HSD17B7 and HSD17B12 may also catalyze progesterone deactivation to 20 α -dihydroprogesterone and conversion of inactive estrone to bioactive estradiol (Li *et al.* 2005, Peltoketo *et al.* 1999, Penning *et al.* 2001, Sakurai *et al.* 2006). AKR1C3 is a pluripotent, widely distributed enzyme catalyzing the conversion of aldehydes and ketones to alcohols (Matsuura *et al.* 1998, Penning *et al.* 2006). This isoform functions as a bi-directional 3 α -, 17 β - and 20 α -HSD and can interconvert active androgens, estrogens and progestins with their cognate biologically inactive metabolites, however, like other AKR1Cs *in vivo*, AKR1C3 preferentially works as a reductase (Matsuura *et al.* 1998, Penning *et al.* 2001, Steckelbroeck *et al.* 2004). Although the AKR1C3 is also expressed in placenta, its importance appears to be secondary to the HSD17B1.

In contrast to the enzymes favoring reductive conversions, the HSD17B2 prefers the oxidative direction catalyzing the progesterone biosynthesis from biologically inactive 20 α -dihydroprogesterone as well as the conversion of bioactive estradiol to biologically inactive estrone (Moghrabi *et al.* 1997). HSD17B2 may also convert the GABAergic 3 α -hydroxy-5 α/β - C21 steroids to inactive and antagonistic substances but, at the same time, may transform the less active GABAergic 3 α ,20 α -dihydroxy-5 α/β -isomers to more active 3 α -hydroxy-5 α/β -20-oxo-isomers.

The site of expression of HSD17B2 was identified in endothelial cells of fetal capillaries and some stem villous vessels (Moghrabi *et al.* 1997, Takeyama *et al.* 1998) and in endothelial cells of villous arteries and arterioles (Bonenfant *et al.* 2000a). Moghrabi *et al.* suggested a protective role of the HSD17B2 from the excess of bioactive estrogens and androgens in the fetus (Moghrabi *et al.* 1997). Besides HSD17B2, the type 14 17 β -HSD (HSD17B14) a member of SDRs may also convert estradiol to estrone and 5-androstene-3 β ,17 β -diol to DHEA (Lukacik *et al.* 2007).

The reversible oxido-reductive interconversion of GABAergic C21 and C19 3 α -hydroxy-5 α/β -reduced metabolites to the corresponding inactive 3-oxo-metabolites and antagonistic 3 β -hydroxy-metabolites (Lundgren *et al.* 2003) may influence the balance between inhibitory and excitatory steroids. While the reductive conversion in the C3 position produce GABAergic steroids, the conversion of 20-oxo- to 20 α -hydroxy-

group or a modification of the C17,20 side chain in the 3 α -hydroxy-5 α/β C21 steroids results in subtype dependent reduction of positive allosteric modulation of GABA_A-r (Belelli *et al.* 1996).

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 and mainly in the liver) and MV. Indeed, the blood from UV actually contains higher proportions of 20-oxo-steroids like progesterone, 17-oxo steroids (e.g. estrone and DHEA), 3-oxo-steroids like 5 α/β -DHP and 3 β -hydroxy-steroids (isopregnanolone and epipregnanolone), while maternal venous blood contains higher proportions of 20 α -hydroxy-steroids like 20 α -dihydroprogesterone, 17 β -hydroxy-steroids (estradiol) and androstenediol and 3 α -hydroxy-5 α/β -reduced steroids like GABAergic allopregnanolone and pregnanolone (Figure 2). Also the levels of conjugated 3 α -hydroxy-5 α/β -reduced-17-oxo C-19 steroids in MV are pronouncedly higher than in the fetal circulation and amniotic fluid, while the 3 β -isomers do not significantly differ (Figure 2) (Hill *et al.* 2010a).

Some authors reported that the metabolism of placental sex steroids in the reductive direction increases as pregnancy advances and significantly rises during human parturition (Diaz-Zagoya *et al.* 1979, Milewich *et al.* 1978). This phenomenon may be important for the initiation of labor and indicate a mechanism of progesterone withdrawal and estradiol rise in association with the onset of human parturition. However, our recent data are ambiguous (Hill *et al.* 2010a).

3 Levels of 5 α/β -reduced progesterone metabolites steroids in pregnant women and fetuses

3.1 Progesterone and its 5 α/β -reduced metabolites in pregnant and non-pregnant women

In pregnant women, there are persistently elevated levels of pregnanolone isomers (PI) including the GABAergic 3 α -PI (Hill *et al.* 2007, Hill *et al.* 2010c, Kancheva *et al.* 2007, Luisi *et al.* 2000, Mickan and Zander 1979, Parizek *et al.* 2005, Pearson Murphy *et al.* 2001) (Table 1). The concentrations in women after luteo-placental shift reach the values about two orders of magnitude higher than the concentrations detected in the follicular phase (Parizek *et al.* 2005). The levels of all of the C21 steroids including their 5 α/β -reduced metabolites rise greatly during pregnancy, being highest for progesterone (562-fold the follicular level), 5 α -DHP

(161-fold), isopregnanolone (56-fold), allopregnanolone (37-fold), pregnenolone (30-fold), 5 β -DHP (16-fold) and epipregnanolone (16-fold) at 37th week of gestation (Luisi *et al.* 2000, Parizek *et al.* 2005, Pearson Murphy *et al.* 2001). These conditions induce a decreased affinity of GABA_A-r for these NAS either due to the changed expression of the receptor subunits and/or as a result of the changed phosphorylation status of the specific sites on the GABA_A-r (Brussaard *et al.* 1997, Koksma *et al.* 2003).

3 α - and 3 β -PI in human circulation strongly correlate irrespectively of sex, menstrual- or pregnancy status (Kancheva *et al.* 2007), which illustrates an uncomplicated, reversible oxidoreductive shift between the GABAergic 3 α -PI and antagonistic 3 β -PI via the inactive 3-oxo-intermediate. Sulfation counteracts the effect of 3 α -PI on GABA_A-r and further amplifies the antagonistic effect of 3 β -PI, forming products that negatively modulate GABA_A-r on the binding sites, which are different from the sites specific to the unconjugated 3 α -PI. The modulation efficiencies of the conjugated neurosteroids on GABA_A-r may reach about 1/10 of those for the corresponding unconjugated substances (Park-Chung *et al.* 1999). Nonetheless, in maternal circulation the concentrations of conjugated pregnane steroids are about 2 orders of magnitude higher when compared with their unconjugated analogues.

3.2 Changing profiles of the circulating 5 α /6-reduced progesterone metabolites during pregnancy

The increasing trend of PI during pregnancy is more distinct for the GABAergic 3 α -PI (Parizek *et al.* 2005) and their polar conjugates increase even more pronouncedly (Hill *et al.* 2007, Hill *et al.* 2010c). These negative modulators of GABA_A-r are present in excessive concentrations in maternal circulation when compared to the free, GABAergic 3 α -PI (Park-Chung *et al.* 1999). Rising levels of all conjugated PI and ratios of all conjugates to the respective free steroids during the 3rd trimester indicate increasing liver sulfotransferase activity for all PI (Hill *et al.* 2007, Hill *et al.* 2010a, Hill *et al.* 2010c). The ratios of conjugated 5 α -PI conjugates to their free counterparts show a gradual increase up to the 37th week, while in the case of 5 β -PI these ratios exhibit an accelerating increase from the 31st week to term. From the PI, the most prominent avidity for conjugation shows the GABAergic pregnanolone. This steroid is chemically identical with the short-term intravenous anesthetic eltanolone (Hering *et al.* 1996, Kallela *et al.* 1994). The conjugation of pregnenolone turns its positive GABAergic effect to the negative one (Park-Chung *et al.* 1999) but, at the same time, produces high amounts of negative modulator of N-methyl-D-aspartate receptors (NMDA-r).

While the levels of unconjugated 5 α / β -reduced progesterone metabolites including PI slightly correlate with GA in the third trimester, the concentrations of conjugated PI display strong positive correlations with the GA although these correlations are still less pronounced than those for the conjugated sulfated Δ^5 steroids or estrogens (Hill *et al.* 2010c).

In the study by Gilbert Evans *et al.*, allopregnanolone and pregnanolone, as well as progesterone, 5 α -DHP, 5 β -DHP, isopregnanolone and pregnenolone, in plasma from healthy women increase significantly from 10th to 36th week of pregnancy, while the 5 β -DHP and pregnenolone do not change significantly (Gilbert Evans *et al.* 2005). Our previous study demonstrates that the ratio 5 α /5 β -PI decrease between the 1st and the 2nd months, stagnates from the 2nd to the 8th months and significantly increases from the 8th to the 10th months, which allows speculating whether these findings are connected to the changing activities of SRD5A and/or AKR1D1 during pregnancy. Whereas the overall SRD5A1 activity shows no alterations during gestation and progesterone still increases or stagnates, the rising 5 α /5 β -PI ratio near term may indicate decreasing AKR1D1 activity (Parizek *et al.* 2005). The turnovers of 5 α -DHP/progesterone and 5 β -DHP/progesterone in the 3rd trimester show that the metabolism of progesterone to 5 α -DHP inconspicuously culminates in the 35th week, while the conversion of progesterone to 5 β -DHP significantly declines from the 31st week of gestation (Hill *et al.* 2007). This is in accordance with results of other authors as well as with our recent data (Gilbert Evans *et al.* 2005, Hill *et al.* 2010a, Hill *et al.* 2010c, Sheehan 2006, Sheehan *et al.* 2005).

The 3 α -hydroxysteroid oxidoreductase-mediated turnovers of 5 α -DHP and 5 β -DHP to allopregnanolone and pregnanolone, respectively, rise during pregnancy, but the turnover of 5 α -DHP to allopregnanolone drops at the late prenatal visit. At 6 weeks postpartum all steroids significantly drop when compared with late prenatal values (Gilbert Evans *et al.* 2005). Although in our previous study we have found no significant change of the ratio 3 α /3 β -PI during pregnancy (Parizek *et al.* 2005), latter on we have found a mild shift from the 3 α -PI to the 3-oxo- and 3 β -isomers (Hill *et al.* 2010a). Gilbert Evans *et al.* show significant but inconsistent changes in PI in the late pregnancy. The authors reported an oxidative shift from allopregnanolone to 5 α -DHP but reductive one from 5 β -DHP to pregnanolone (Gilbert Evans *et al.* 2005).

3.3 Changes of circulating progesterone and their 5 α / β -reduced metabolites around parturition

Progesterone and 20 α -dihydroprogesterone levels decrease during labor (Nakayama 1986). On the other hand, progesterone levels in serial plasma samples from maternal blood collected during the last eight weeks of gestation and the samples collected during the second stage of labor do not significantly differ (Mathur *et al.* 1980).

Lofgren & Backström following the levels of progesterone and 5 α -DHP in 11 women with uncomplicated pregnancies and deliveries during spontaneous labor and immediately after delivery demonstrated no significant changes during labor in serum concentration of progesterone and 5 α -DHP, whereas their earlier study reported a significant decrease in 5 α -DHP serum concentration between late pregnancy and spontaneous labor. Nevertheless, the authors suggested that the decrease occurs already before the onset of labor. About 2-3 hours post partum the steroid levels stabilized just above luteal phase values. 12 h post partum, progesterone and 5 α -DHP were 12% and 23% respectively of pre-partum values (Lofgren and Backstrom 1990).

Pearson Murphy *et al.* demonstrated that during the period 2-7 day postpartum, the level of progesterone fell precipitously, whereas those of pregnenolone and the metabolites decrease more slowly and mean levels were still elevated compared with follicular levels 2 weeks after delivery. By the 7th week postpartum only allopregnanolone and epipregnanolone remained slightly elevated (Pearson Murphy *et al.* 2001). Our recent report (Hill *et al.* 2010a, Hill *et al.* 2010c) as well as our previous data for PI around parturition display significantly lower ratios of conjugated PI to their unconjugated counterparts in the umbilical venous plasma than in the maternal plasma (Hill *et al.* 2001, Klak *et al.* 2003). Changes in concentrations of individual free PI in the maternal serum exhibit a similar pattern, with a fall mostly within the first hour after delivery. The decrease in conjugated steroids is shifted to the interval within the first hour and first day after delivery (Hill *et al.* 2001, Klak *et al.* 2003). The conjugated/free steroid ratios significantly decrease within the first hour and the first day after delivery in all PI (Hill *et al.* 2001, Klak *et al.* 2003). These results point to an intensive sulfation of GABAergic substances in the maternal compartment during pregnancy but attenuating sulfation activity postpartum and show that the sulfation of GABAergic steroids (transforming them to their antagonists) may represent a mechanism counterbalancing their overproduction in pregnant women.

The ratios of 3 α - to the 3 β -PI decrease around parturition (Hill *et al.* 2001, Klak *et al.* 2003). This finding indicates that the placental and perhaps also the liver reductive conversion of the 3-oxo- and 3 β -hydroxy-5 α / β -PI to the 3 α -isomers are important for the pregnancy sustaining.

4 Effects of 5 α / β -reduced C21 steroids in pregnant women and fetuses

4.1 The role of 5 α / β -reduced progesterone metabolites in pregnancy sustaining and induction of labor

The role of the most abundant PI allopregnanolone in the onset of parturition has been reported in rats where a positive feedback loop in oxytocin production resulting in a rapid delivery forms just before labor. A decrease of allopregnanolone levels triggers the production of oxytocin (Brussaard *et al.* 1997, Koksma *et al.* 2003). This substance brings the GABA_A-r from a neurosteroid-sensitive mode towards a condition, in which the receptors are not sensitive (Koksma *et al.* 2003), via a shift in the balance between the activities of endogenous Ser/Thr phosphatase and protein kinase C (Koksma *et al.* 2003).

On peripheral level, the stimulation of GABA_A-r tonically inhibits contractions of rabbit uterine strips, while the stimulation of GABA_B receptors enhances uterine contractions. Steroids may interact with GABA_A-r to modulate uterine contractility: allopregnanolone inhibits while pregnenolone sulfate increases the contractions. Allopregnanolone rapidly antagonizes the stimulatory effect of pregnenolone sulfate, but progesterone inhibits the contractions after a delay, suggesting that the known pregnancy sustaining effect of progesterone on the uterus could be at least partly mediated via the progesterone metabolite allopregnanolone, which potentiates the inhibitory function of GABA_A-r (Majewska and Vaupel 1991). Contrary, Lofgren *et al.* (Lofgren *et al.* 1992) reported that 5 α -reduced progesterone metabolites are not potent inhibitors of contracting human myometrium.

In recent study on rats, the authors (Fujii and Mellon 2001) found a new subunit of the GABA_A-r, π , being particularly abundant in the rat uterus. Allopregnanolone modulates GABA_A-r activity and neuronal inhibition by modulating the frequency and duration of GABA_A channel opening. This modulation depends on the specific subunit composition of the GABA_A-r. Assembly of recombinant π and δ GABA_A-r subunits into a functional GABA_A-r have been reported to reduce sensitivity to allopregnanolone. As allopregnanolone works through the GABA_A-r to reduce uterine contractions, the researchers hypothesized that incorporation of the π -subunit into

this receptor in the uterus might change the sensitivity of the GABA_A-r to allopregnanolone and modulate parturition. GABA_A π -subunit mRNA abundance was constant throughout gestation, but decreased at the onset of labor, while other GABA_A subunits fluctuated differently during pregnancy: GABA_A α_1 -subunit mRNA expression increased, α_2 - and δ -subunit mRNA expression decreased during pregnancy, and β_3 -subunit mRNA only appeared on postpartum day 1.

Pregnant women and fetuses have exceedingly elevated levels of steroids, which positively modulate NMDA-r, like the sulfated Δ^5 steroids and sulfates of 5 α -PI (Hill *et al.* 2007, Hill *et al.* 2010a, Hill *et al.* 2010c, Malayev *et al.* 2002, Weaver *et al.* 2000). On the other hand, the sulfated 5 β -PI, pregnanolone exerts antagonistic effect on the NMDA-r (Malayev *et al.* 2002, Park-Chung *et al.* 1997, Weaver *et al.* 2000) and promotes their desensitization (Kussius *et al.* 2009). The levels of one of the conjugated 5 β -PI, pregnanolone, are also extremely elevated in pregnant women and show the most prominent rise in the late pregnancy in spite of declining levels of unconjugated pregnanolone (Hill *et al.* 2007, Hill *et al.* 2010c).

Catabolizing both 5 β -reduced steroids providing uterine quiescence and allopregnanolone that relaxes myometrium through voltage-dependent K⁺ channels (Perusquia and Jasso-Kamel 2001) or via GABA_A-r modulation, sulfation may shift the biological activity towards induction of labor.

While progesterone and its 5 α / β -reduced metabolites induce opening of the voltage-dependent K⁺ channels, estradiol is their antagonist (Knock *et al.* 2001, Yoshihara *et al.* 2005). Therefore the ratios progesterone/estradiol and 3 α -hydroxy-5 α / β -pregnane-steroids/estradiol may be of importance for sustaining the uterine quiescence. Whereas allopregnanolone stagnates from the 36th week of gestation (Hill *et al.* 2007), estradiol (Buster *et al.* 1979, Parizek *et al.* 2005, Turnbull *et al.* 1974) still shows an increasing trend. These findings point to a shift of the steroid metabolome from the pregnancy protracting conditions to the parturition provoking state.

When evaluating the capacity to inhibit the *in vitro* motility of rat uterus, progestins with their ring A reduced in the 5 β -position are significantly more potent than the progesterone and 5 α -reduced progestins (Kubli-Garfias *et al.* 1979, Perusquia and Jasso-Kamel 2001). The progestins elicit an immediate relaxing effect that is dose-dependent. The potency order is: allopregnanolone > 5 β -DHP > epipregnanolone > pregnanolone > progesterone > 5 α -DHP > isopregnanolone. This rapid and reversible relaxing effect is not blocked by antiprogestin RU 486, which suggests its independence of receptor-mediated genomic action (Perusquia and

Jasso-Kamel 2001). Besides the modulation of ionotropic receptors, 5 β -reduced metabolites of progesterone may act in pregnancy through a mechanism mediated by pregnane X-type receptors (Mitchell *et al.* 2005, Putnam *et al.* 1991).

The aforementioned effects of 5 α/β -reduced progesterone metabolites, a pronounced decrease of the ratio of conjugated PI to free PI after delivery (Hill *et al.* 2001), accelerating sulfation of pregnancy-sustaining PI and decreasing activity of AKR1D1 from the 31st week of gestation at physiologically relevant concentrations of 5 α/β -reduced progesterone metabolites in late pregnancy allow to speculate as to whether these changes might influence the sustaining of human pregnancy (Gilbert Evans *et al.* 2005, Hill *et al.* 2007, Hill *et al.* 2010a, Hill *et al.* 2010c, Sheehan 2006).

4.2 Effects of neuroactive pregnane metabolites on pain perception, induction of tolerance, receptor plasticity

4.2.1 The effects of neuroactive pregnane metabolites in the fetal CNS

Allopregnanolone may interact with GABA_A-r to inhibit fetal CNS activity from mid-gestation. This inhibition may contribute to maintaining the sleep-like behavior and low incidence of arousal-type activity typical of fetal life (Crossley *et al.* 2003). Some authors (Mellor *et al.* 2005) suggest that the uterus plays a key role in providing chemical and physical factors that keep the fetus continuously asleep. The mechanism providing the permanent sleeping status in the fetus combines neuroinhibitory actions of a powerful EEG suppressor and sleep inducing agent (adenosine), two GABAergic steroids anesthetics (allopregnanolone, pregnanolone) and a potent sleep-inducing hormone (prostaglandin D2), acting together with a putative peptide inhibitor and other placental factors (Mellor *et al.* 2005).

Concerning the role of GABAergic steroids in suppressing the nociceptive pathways in the fetus, our data (Hill *et al.* 2010a) shows 2-3 times lower allopregnanolone levels in the fetal circulation than in the maternal one, while the pregnanolone levels in UV exceed those in MV 1-2.5 times. The total amount of GABAergic PI is only slightly higher in the fetal compartment, predominantly due to contribution of unconjugated pregnanolone. Therefore peripheral GABAergic steroids exert a comparable effect on the maternal and fetal CNS. Even when considering the 1.5-3 fold excess of progesterone in the fetal circulation (when compared to maternal blood),

progesterone transport into the brain and its subsequent conversion to the GABAergic steroids, the resulting contribution of GABAergic steroids originating from peripheral sources do not pronouncedly differ between mother and fetus. Therefore the importance of GABAergic steroids for maintenance of permanent fetal sleeping is open to discussion.

4.2.2 *The effects of neuroactive pregnane metabolites in the maternal CNS*

The increased brain levels of NAS during pregnancy are causally related to changing the expression of specific GABA_A-r subunits in the cerebral cortex and hippocampus (Concas *et al.* 1999, Mostallino *et al.* 2009). Allopregnanolone treatment in mice induces a partial tolerance against acute allopregnanolone effects (Turkmen *et al.* 2006). Alterations in δ GABA_A-r subunit expression during pregnancy result in brain region-specific increases in neuronal excitability that are restored by high allopregnanolone levels under normal conditions but under pathological conditions may induce neurological and psychiatric disorders associated with pregnancy and postpartum period (Maguire *et al.* 2009). In all probability, the changes in neuronal excitability during pregnancy are attributable to the alterations in expression of δ GABA_A-r subunit (Maguire *et al.* 2009).

Besides the GABAergic effects in the CNS and periphery, 5 β -reduced progesterone metabolites also exert peripheral analgesic effects via blockade of calcium channels controlling pain perception (Todorovic *et al.* 2004). The data indicates that these steroids might operate as endogenous analgesics around parturition.

4.3 ***Neuroprotective and excitotoxic effects of 5 α / β -reduced pregnanes***

Although the neurosteroids modulating GABAergic activity may be synthesized *de novo* within the fetal brain, some studies indicate an association between concentrations of NAS in the brain, the activity of FZ and placental progesterone production. Fetuses exposed to stress during labor produce higher progesterone, which may protect them against 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 FZ.

Physiologic concentrations of allopregnanolone protect against NMDA induced excitotoxicity via positive modulation of GABA_A-r (Crossley *et al.* 2003, Lockhart *et al.* 2002). Growth restriction is a potent stimulus for neurosteroid synthesis in the fetal brain in late pregnancy. Inhibition of allopregnanolone synthesis by finasteride, a SRD5A type 2 inhibitor, results in increasing constitutive rate of apoptosis and proliferation of

pyknotic cells of fetal hippocampus and cerebellum (Yawno *et al.* 2009). Although allopregnanolone levels are high in the fetal brain, they raise further in response to acute hypoxic stress. This response may result from the increased SRD5A and CYP11A1 expression in the brain as suggested by Hirst *et al.* (Hirst *et al.* 2006) and from the increased stress-induced peripheral production of progesterone.

5 Summary

The data summarized in this review shows that besides the progesterone, a variety of its 5 α / β -reduced metabolites may significantly influence the stability of human pregnancy, provide protection against excitotoxicity following acute hypoxic stress, and might also affect the pain perception in mother and fetus.

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7 References

ANTONIPILLAI I, MURPHY BE: Serum oestrogens and progesterone in mother and infant at delivery. *Br J Obstet Gynaecol* **84**: 179-85, 1977.

BELELLI D, LAMBERT JJ, PETERS JA, GEE KW, LAN NC: Modulation of human recombinant GABAA receptors by pregnanediols. *Neuropharmacology* **35**: 1223-31, 1996.

BONENFANT M, BLOMQUIST CH, PROVOST PR, DROLET R, D'ASCOLI P, TREMBLAY Y: Tissue- and site-specific gene expression of type 2 17 β -hydroxysteroid dehydrogenase: in situ hybridization and specific enzymatic activity studies in human placental endothelial cells of the arterial system. *J Clin Endocrinol Metab* **85**: 4841-50, 2000a.

BONENFANT M, PROVOST PR, DROLET R, TREMBLAY Y: Localization of type 1 17 β -hydroxysteroid dehydrogenase mRNA and protein in syncytiotrophoblasts and invasive cytotrophoblasts in the human term villi. *J Endocrinol* **165**: 217-22, 2000b.

BRERETON P, SUZUKI T, SASANO H, LI K, DUARTE C, OBEYESEKERE V, HAESELEER F, PALCZEWSKI K, SMITH I, KOMESAROFF P, KROZOWSKI Z: Pan1b (17betaHSD11)-enzymatic activity and distribution in the lung. *Mol Cell Endocrinol* **171**: 111-7, 2001.

BRUSSAARD AB, KITS KS, BAKER RE, WILLEMS WP, LEYTING-VERMEULEN JW, VOORN P, SMIT AB, BICKNELL RJ, HERBISON AE: Plasticity in fast synaptic inhibition of adult oxytocin neurons caused by switch in GABA(A) receptor subunit expression. *Neuron* **19**: 1103-14, 1997.

BUSTER JE, CHANG RJ, PRESTON DL, ELASHOFF RM, COUSINS LM, ABRAHAM GE, HOBEL CJ, MARSHALL JR: Interrelationships of circulating maternal steroid concentrations in third trimester pregnancies. II. C18 and C19 steroids: estradiol, estriol, dehydroepiandrosterone, dehydroepiandrosterone sulfate, delta 5-androstenediol, delta 4-androstenedione, testosterone, and dihydrotestosterone. *J Clin Endocrinol Metab* **48**: 139-42, 1979.

CONCAS A, FOLLESA P, BARBACCIA ML, PURDY RH, BIGGIO G: Physiological modulation of GABA(A) receptor plasticity by progesterone metabolites. *Eur J Pharmacol* **375**: 225-35, 1999.

CROSSLEY KJ, NITSOS I, WALKER DW, LAWRENCE AJ, BEART PM, HIRST JJ: Steroid-sensitive GABAA receptors in the fetal sheep brain. *Neuropharmacology* **45**: 461-72, 2003.

DIAZ-ZAGOYA JC, WIEST WG, ARIAS F: 20 alpha-Hydroxysteroid oxidoreductase activity and 20 alpha-dihydroprogesterone concentration in human placenta before and after parturition. *Am J Obstet Gynecol* **133**: 673-6, 1979.

FUJII E, MELLON SH: Regulation of uterine gamma-aminobutyric acid(A) receptor subunit expression throughout pregnancy. *Endocrinology* **142**: 1770-7, 2001.

FUKUDA M, OKUYAMA T, FURUYA H: Growth and function of the placenta--with special reference to various enzymes involved in the biosynthesis of steroids in the human placenta. *Nippon Sanka Fujinka Gakkai Zasshi* **38**: 411-6, 1986.

GILBERT EVANS SE, ROSS LE, SELLERS EM, PURDY RH, ROMACH MK: 3alpha-reduced neuroactive steroids and their precursors during pregnancy and the postpartum period. *Gynecol Endocrinol* **21**: 268-79, 2005.

GOLAND RS, WARDLAW SL, STARK RI, BROWN LS, JR., FRANTZ AG: High levels of corticotropin-releasing hormone immunoactivity in maternal and fetal plasma during pregnancy. *J Clin Endocrinol Metab* **63**: 1199-203, 1986.

HAWES CS, MCBRIDE MW, PETROPOULOS A, MUELLER UW, SUTCLIFFE RG: Epitopic heterogeneity of human 3 beta-hydroxysteroid dehydrogenase in villous and extravillous human trophoblast. *J Mol Endocrinol* **12**: 273-81, 1994.

HE XY, MERZ G, YANG YZ, MEHTA P, SCHULZ H, YANG SY: Characterization and localization of human type 10 17beta-hydroxysteroid dehydrogenase. *Eur J Biochem* **268**: 4899-907, 2001.

HE XY, YANG YZ, PEEHL DM, LAUDERDALE A, SCHULZ H, YANG SY: Oxidative 3alpha-hydroxysteroid dehydrogenase activity of human type 10 17beta-hydroxysteroid dehydrogenase. *J Steroid Biochem Mol Biol* **87**: 191-8, 2003.

HERING WJ, IHMSEN H, LANGER H, UHRLAU C, DINKEL M, GEISLINGER G, SCHUTTLER J: Pharmacokinetic-pharmacodynamic modeling of the new steroid hypnotic eltanolone in healthy volunteers. *Anesthesiology* **85**: 1290-9, 1996.

HILL M, BICIKOVA M, PARIZEK A, HAVLIKOVA H, KLAK J, FAJT T, MELOUN M, CIBULA D, CEGAN A, SULCOVA J, HAMPL R, STARKA L: Neuroactive steroids, their precursors and polar conjugates during parturition and postpartum in maternal blood: 2. Time profiles of pregnanolone isomers. *J Steroid Biochem Mol Biol* **78**: 51-7, 2001.

HILL M, CIBULA D, HAVLIKOVA H, KANCHEVA L, FAJT T, KANCHEVA R, PARIZEK A, STARKA L: Circulating levels of pregnanolone isomers during the third trimester of human pregnancy. *J Steroid Biochem Mol Biol* **105**: 166-75, 2007.

HILL M, PARIZEK A, BICIKOVA M, HAVLIKOVA H, KLAK J, FAJT T, CIBULA D, HAMPL R, CEGAN A, SULCOVA J, STARKA L: Neuroactive steroids, their precursors, and polar conjugates during parturition and postpartum in maternal and umbilical blood: 1. Identification and simultaneous determination of pregnanolone isomers. *J Steroid Biochem Mol Biol* **75**: 237-44, 2000.

HILL M, PARIZEK A, CIBULA D, KANCHEVA R, JIRASEK JE, JIRKOVSKA M, VELIKOVA M, KUBATOVA J, KLIMKOVA M, PASKOVA A, ZIZKA Z, KANCHEVA L, KAZIHNITKOVA H, ZAMRAZILOVA L, STARKA L: Steroid metabolome in fetal and maternal body fluids in human late pregnancy. *J Steroid Biochem Mol Biol* 2010a.

HILL M, PARIZEK A, JIRASEK JE, JIRKOVSKA M, VELIKOVA M, DUSKOVA M, KLIMKOVA M, PASKOVA A, ZIZKA Z, GERMANOVA A, KOUCKY M, KALOUSOVA M, STARKA L: Is maternal progesterone actually independent of the fetal steroids? *Physiol Res* **59**: 211-24, 2010b.

HILL M, PARIZEK A, KANCHEVA R, DUSKOVA M, VELIKOVA M, KRIZ L, KLIMKOVA M, PASKOVA A, ZIZKA Z, MATUCHA P, MELOUN M, STARKA L: Steroid metabolome in plasma from the umbilical artery, umbilical vein, maternal cubital vein and in amniotic fluid in normal and preterm labor. *J Steroid Biochem Mol Biol* **121**: 594-610, 2010c.

HIRST JJ, YAWNO T, NGUYEN P, WALKER DW: Stress in pregnancy activates neurosteroid production in the fetal brain. *Neuroendocrinology* **84**: 264-74, 2006.

HUANG XF, LUU-THE V: Molecular characterization of a first human 3(alpha-->beta)-hydroxysteroid epimerase. *J Biol Chem* **275**: 29452-7, 2000.

CHAI Z, BRERETON P, SUZUKI T, SASANO H, OBEYESEKERE V, ESCHER G, SAFFERY R, FULLER P, ENRIQUEZ C, KROZOWSKI Z: 17 beta-hydroxysteroid dehydrogenase type XI localizes to human steroidogenic cells. *Endocrinology* **144**: 2084-91, 2003.

CHARBONNEAU A, THE VL: Genomic organization of a human 5beta-reductase and its pseudogene and substrate selectivity of the expressed enzyme. *Biochim Biophys Acta* **1517**: 228-35, 2001.

INGELMAN-SUNDBERG M, RANE A, GUSTAFASSON JA: Properties of hydroxylase systems in the human fetal liver active on free and sulfoconjugated steroids. *Biochemistry* **14**: 429-37, 1975.

ISHIDA T, SEO F, HIRATO K, FUKUDA T, YANAIHARA T, ARAKI H, NAKAYAMA T: [Changes in placental enzymatic activities in relation to estrogen production during pregnancy]. *Nippon Sanka Fujinka Gakkai Zasshi* **37**: 547-54, 1985.

JAFFE RB, LEDGER WJ: In vivo steroid biogenesis and metabolism in the human term placenta. I. In situ placental perfusion with isotopic pregnenolone. *Steroids* **8**: 61-78, 1966.

JIN Y, DUAN L, LEE SH, KLOOSTERBOER HJ, BLAIR IA, PENNING TM: Human cytosolic hydroxysteroid dehydrogenases of the aldo-ketoreductase superfamily catalyze reduction of conjugated steroids: implications for phase I and phase II steroid hormone metabolism. *J Biol Chem* **284**: 10013-22, 2009.

JIN Y, PENNING TM: Steroid 5 α -reductases and 3 α -hydroxysteroid dehydrogenases: key enzymes in androgen metabolism. *Best Pract Res Clin Endocrinol Metab* **15**: 79-94, 2001.

JIN Y, STAYROOK SE, ALBERT RH, PALACKAL NT, PENNING TM, LEWIS M: Crystal structure of human type III 3 α -hydroxysteroid dehydrogenase/bile acid binding protein complexed with NADP(+) and ursodeoxycholate. *Biochemistry* **40**: 10161-8, 2001.

KALLELA H, HAASIO J, KORTTILA K: Comparison of etanolone and propofol in anesthesia for termination of pregnancy. *Anesth Analg* **79**: 512-6, 1994.

KANCHEVA R, HILL M, CIBULA D, VCELAKOVA H, KANCHEVA L, VRBIKOVA J, FAIT T, PARIZEK A, STARKA L: Relationships of circulating pregnanolone isomers and their polar conjugates to the status of sex, menstrual cycle, and pregnancy. *J Endocrinol* **195**: 67-78, 2007.

KLAK J, HILL M, PARIZEK A, HAVLIKOVA H, BICIKOVA M, HAMPL R, FAIT T, SULCOVA J, POUZAR V, KANCHEVA R, STARKA L: Pregnanolone isomers, pregnenolone and their polar conjugates around parturition. *Physiol Res* **52**: 211-21, 2003.

KNOCK GA, TRIBE RM, HASSONI AA, AARONSON PI: Modulation of potassium current characteristics in human myometrial smooth muscle by 17 β -estradiol and progesterone. *Biol Reprod* **64**: 1526-34, 2001.

KOCHAKIAN CD: Conversion of testosterone and androstenedione to 5 β -androstanes by adult male hamster liver cytosol. *J Steroid Biochem* **19**: 1521-6, 1983.

KOKSMA JJ, VAN KESTEREN RE, ROSAHL TW, ZWART R, SMIT AB, LUDDENS H, BRUSSAARD AB: Oxytocin regulates neurosteroid modulation of GABA(A) receptors in supraoptic nucleus around parturition. *J Neurosci* **23**: 788-97, 2003.

KOMATSUZAKI K, KOSAKI T, HASHINO M, YANAIHARA T, NAKAYAMA T, MORI H: [Metabolism of pregnenolone sulfate in feto-placental unit]. *Nippon Sanka Fujinka Gakkai Zasshi* **39**: 1095-102, 1987.

KRAZEISEN A, BREITLING R, IMAI K, FRITZ S, MOLLER G, ADAMSKI J: Determination of cDNA, gene structure and chromosomal localization of the novel human 17beta-hydroxysteroid dehydrogenase type 7(1). *FEBS Lett* **460**: 373-9, 1999.

KUBLI-GARFIAS C, MEDRANO-CONDE L, BEYER C, BONDANI A: In vitro inhibition of rat uterine contractility induced by 5 alpha and 5 beta progestins. *Steroids* **34**: 609-17, 1979.

KUSSIUS CL, KAUR N, POPESCU GK: Pregnanolone sulfate promotes desensitization of activated NMDA receptors. *J Neurosci* **29**: 6819-27, 2009.

LACROIX D, SONNIER M, MONCION A, CHERON G, CRESTEIL T: Expression of CYP3A in the human liver--evidence that the shift between CYP3A7 and CYP3A4 occurs immediately after birth. *Eur J Biochem* **247**: 625-34, 1997.

LEEDER JS, GAEDIGK R, MARCUCCI KA, GAEDIGK A, VYHLIDAL CA, SCHINDEL BP, PEARCE RE: Variability of CYP3A7 expression in human fetal liver. *J Pharmacol Exp Ther* **314**: 626-35, 2005.

LESLIE KK, ZUCKERMAN DJ, SCHRUEFER J, BURCHELL M, SMITH J, ALBERTSON BD: Oestrogen modulation with parturition in the human placenta. *Placenta* **15**: 79-88, 1994.

LI Y, ISOMAA V, PULKKA A, HERVA R, PELTOKETO H, VIHKO P: Expression of 3beta-hydroxysteroid dehydrogenase type 1, P450 aromatase, and 17beta-hydroxysteroid dehydrogenase types 1, 2, 5 and 7 mRNAs in human early and mid-gestation placentas. *Placenta* **26**: 387-92, 2005.

LIN SX, SHI R, QIU W, AZZI A, ZHU DW, DABBAGH HA, ZHOU M: Structural basis of the multispecificity demonstrated by 17beta-hydroxysteroid dehydrogenase types 1 and 5. *Mol Cell Endocrinol* **248**: 38-46, 2006.

LOCKHART EM, WARNER DS, PEARLSTEIN RD, PENNING DH, MEHRABANI S, BOUSTANY RM: Allopregnanolone attenuates N-methyl-D-aspartate-induced excitotoxicity and apoptosis in the human NT2 cell line in culture. *Neurosci Lett* **328**: 33-6, 2002.

LOFGREN M, BACKSTROM T: Serum concentrations of progesterone and 5 alpha-pregnane-3,20-dione during labor and early post partum. *Acta Obstet Gynecol Scand* **69**: 123-6, 1990.

LOFGREN M, HOLST J, BACKSTROM T: Effects in vitro of progesterone and two 5 alpha-reduced progestins, 5 alpha-pregnane-3,20-dione and 5 alpha-pregnane-3 alpha-ol-20-one, on contracting human myometrium at term. *Acta Obstet Gynecol Scand* **71**: 28-33, 1992.

LUISI S, PETRAGLIA F, BENEDETTO C, NAPPI RE, BERNARDI F, FADALTI M, REIS FM, LUISI M, GENAZZANI AR: Serum allopregnanolone levels in pregnant women: changes during pregnancy, at delivery, and in hypertensive patients. *J Clin Endocrinol Metab* **85**: 2429-33, 2000.

LUKACIK P, KELLER B, BUNKOCZI G, KAVANAGH KL, LEE WH, ADAMSKI J, OPPERMANN U: Structural and biochemical characterization of human orphan DHRS10 reveals a novel cytosolic enzyme with steroid dehydrogenase activity. *Biochem J* **402**: 419-27, 2007.

LUNDGREN P, STROMBERG J, BACKSTROM T, WANG M: Allopregnanolone-stimulated GABA-mediated chloride ion flux is inhibited by 3beta-hydroxy-5alpha-pregnan-20-one (isosalpregnanolone). *Brain Res* **982**: 45-53, 2003.

MAGUIRE J, FERANDO I, SIMONSEN C, MODY I: Excitability changes related to GABA_A receptor plasticity during pregnancy. *J Neurosci* **29**: 9592-601, 2009.

MAJEWSKA MD, VAUPEL DB: Steroid control of uterine motility via gamma-aminobutyric acid_A receptors in the rabbit: a novel mechanism? *J Endocrinol* **131**: 427-34, 1991.

MALAYEV A, GIBBS TT, FARB DH: Inhibition of the NMDA response by pregnenolone sulphate reveals subtype selective modulation of NMDA receptors by sulphated steroids. *Br J Pharmacol* **135**: 901-9, 2002.

MASON JI, USHIJIMA K, DOODY KM, NAGAI K, NAVILLE D, HEAD JR, MILEWICH L, RAINEY WE, RALPH MM: Regulation of expression of the 3 beta-hydroxysteroid dehydrogenases of human placenta and fetal adrenal. *J Steroid Biochem Mol Biol* **47**: 151-9, 1993.

MATHUR RS, LANDGREBE S, WILLIAMSON HO: Progesterone, 17-hydroxyprogesterone, estradiol, and estriol in late pregnancy and labor. *Am J Obstet Gynecol* **136**: 25-7, 1980.

MATSUURA K, SHIRAIISHI H, HARA A, SATO K, DEYASHIKI Y, NINOMIYA M, SAKAI S: Identification of a principal mRNA species for human 3alpha-hydroxysteroid dehydrogenase isoform (AKR1C3) that exhibits high prostaglandin D2 11-ketoreductase activity. *J Biochem* **124**: 940-6, 1998.

MEIKLE AW, STRINGHAM JD, WILSON DE, DOLMAN LI: Plasma 5 alpha-reduced androgens in men and hirsute women: role of adrenals and gonads. *J Clin Endocrinol Metab* **48**: 969-75, 1979.

MELLOR DJ, DIESCH TJ, GUNN AJ, BENNET L: The importance of 'awareness' for understanding fetal pain. *Brain Res Brain Res Rev* **49**: 455-71, 2005.

MENG LJ, REYES H, AXELSON M, PALMA J, HERNANDEZ I, RIBALTA J, SJOVALL J: Progesterone metabolites and bile acids in serum of patients with intrahepatic cholestasis of pregnancy: effect of ursodeoxycholic acid therapy. *Hepatology* **26**: 1573-9, 1997.

MICKAN H, ZANDER J: Pregnanolones, pregnenolone and progesterone in the human fetoplacental circulation at term of pregnancy. *J Steroid Biochem* **11**: 1461-6, 1979.

MIKI Y, NAKATA T, SUZUKI T, DARNEL AD, MORIYA T, KANEKO C, HIDAKA K, SHIOTSU Y, KUSAKA H, SASANO H: Systemic distribution of steroid sulfatase and estrogen sulfotransferase in human adult and fetal tissues. *J Clin Endocrinol Metab* **87**: 5760-8, 2002.

MILEWICH L, GANT NF, SCHWARZ BE, CHEN GT, MACDONALD PC: Initiation of human parturition. IX. Progesterone metabolism by placentas of early and late human gestation. *Obstet Gynecol* **51**: 278-80, 1978.

—: 5 alpha-Reductase activity in human placenta. *Am J Obstet Gynecol* **133**: 611-7, 1979.

MITCHELL BF, MITCHELL JM, CHOWDHURY J, TOUGAS M, ENGELEN SM, SENFF N, HEIJNEN I, MOORE JT, GOODWIN B, WONG S, DAVIDGE ST: Metabolites of progesterone and the pregnane X receptor: a novel pathway regulating uterine contractility in pregnancy? *Am J Obstet Gynecol* **192**: 1304-13; discussion 1313-5, 2005.

MITCHELL BF, POWELL WA: Progesterone production by human fetal membranes: an in vitro incubation system for studying hormone production and metabolism. *Am J Obstet Gynecol* **148**: 303-9, 1984.

MOGHRABI N, HEAD JR, ANDERSSON S: Cell type-specific expression of 17 beta-hydroxysteroid dehydrogenase type 2 in human placenta and fetal liver. *J Clin Endocrinol Metab* **82**: 3872-8, 1997.

MOSTALLINO MC, SANNA E, CONCAS A, BIGGIO G, FOLLESA P: Plasticity and function of extrasynaptic GABA(A) receptors during pregnancy and after delivery. *Psychoneuroendocrinology* **34 Suppl 1**: S74-83, 2009.

NAKAYAMA T: [Clinical significance of the feto-placental unit during pregnancy and parturition]. *Nippon Sanka Fujinka Gakkai Zasshi* **38**: 1173-83, 1986.

NISHIZAWA M, NAKAJIMA T, YASUDA K, KANZAKI H, SASAGURI Y, WATANABE K, ITO S: Close kinship of human 20alpha-hydroxysteroid dehydrogenase gene with three aldo-keto reductase genes. *Genes Cells* **5**: 111-25, 2000.

OKUDA A, OKUDA K: Purification and characterization of delta 4-3-ketosteroid 5 beta-reductase. *J Biol Chem* **259**: 7519-24, 1984.

PARIZEK A, HILL M, KANCHEVA R, HAVLIKOVA H, KANCHEVA L, CINDR J, PASKOVA A, POUZAR V, CERNY I, DRBOHLAV P, HAJEK Z, STARKA L: Neuroactive pregnanolone isomers during pregnancy. *J Clin Endocrinol Metab* **90**: 395-403, 2005.

PARK-CHUNG M, MALAYEV A, PURDY RH, GIBBS TT, FARB DH: Sulfated and unsulfated steroids modulate gamma-aminobutyric acidA receptor function through distinct sites. *Brain Res* **830**: 72-87, 1999.

PARK-CHUNG M, WU FS, PURDY RH, MALAYEV AA, GIBBS TT, FARB DH: Distinct sites for inverse modulation of N-methyl-D-aspartate receptors by sulfated steroids. *Mol Pharmacol* **52**: 1113-23, 1997.

PEARSON MURPHY BE, STEINBERG SI, HU FY, ALLISON CM: Neuroactive ring A-reduced metabolites of progesterone in human plasma during pregnancy: elevated levels of 5 alpha-dihydroprogesterone in depressed patients during the latter half of pregnancy. *J Clin Endocrinol Metab* **86**: 5981-7, 2001.

PELTOKETO H, NOKELAINEN P, PIAO YS, VIHKO R, VIHKO P: Two 17beta-hydroxysteroid dehydrogenases (17HSDs) of estradiol biosynthesis: 17HSD type 1 and type 7. *J Steroid Biochem Mol Biol* **69**: 431-9, 1999.

PENNING TM: Molecular determinants of steroid recognition and catalysis in aldo-keto reductases. Lessons from 3alpha-hydroxysteroid dehydrogenase. *J Steroid Biochem Mol Biol* **69**: 211-25, 1999.

PENNING TM, BURCZYNSKI ME, JEZ JM, HUNG CF, LIN HK, MA H, MOORE M, PALACKAL N, RATNAM K: Human 3alpha-hydroxysteroid dehydrogenase isoforms (AKR1C1-AKR1C4) of the aldo-keto reductase superfamily: functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex hormones. *Biochem J* **351**: 67-77, 2000.

PENNING TM, BURCZYNSKI ME, JEZ JM, LIN HK, MA H, MOORE M, RATNAM K, PALACKAL N: Structure-function aspects and inhibitor design of type 5 17beta-hydroxysteroid dehydrogenase (AKR1C3). *Mol Cell Endocrinol* **171**: 137-49, 2001.

PENNING TM, JIN Y, STECKELBROECK S, LANISNIK RIZNER T, LEWIS M: Structure-function of human 3 alpha-hydroxysteroid dehydrogenases: genes and proteins. *Mol Cell Endocrinol* **215**: 63-72, 2004.

PENNING TM, STECKELBROECK S, BAUMAN DR, MILLER MW, JIN Y, PEEHL DM, FUNG KM, LIN HK: Aldo-keto reductase (AKR) 1C3: role in prostate disease and the development of specific inhibitors. *Mol Cell Endocrinol* **248**: 182-91, 2006.

PERUSQUIA M, JASSO-KAMEL J: Influence of 5alpha- and 5beta-reduced progestins on the contractility of isolated human myometrium at term. *Life Sci* **68**: 2933-44, 2001.

POWER ML, SCHULKIN J: Functions of corticotropin-releasing hormone in anthropoid primates: from brain to placenta. *Am J Hum Biol* **18**: 431-47, 2006.

PUTNAM CD, BRANN DW, KOLBECK RC, MAHESH VB: Inhibition of uterine contractility by progesterone and progesterone metabolites: mediation by progesterone and gamma amino butyric acidA receptor systems. *Biol Reprod* **45**: 266-72, 1991.

RAINEY WE, REHMAN KS, CARR BR: The human fetal adrenal: making adrenal androgens for placental estrogens. *Semin Reprod Med* **22**: 327-36, 2004.

RILEY SC, BASSETT NS, BERDUSCO ET, YANG K, LEYSTRA-LANTZ C, LUU-THE V, LABRIE F, CHALLIS JR: Changes in the abundance of mRNA for type-I 3 beta-hydroxysteroid dehydrogenase/delta 5-->delta 4 isomerase in the human placenta and fetal membranes during pregnancy and labor. *Gynecol Obstet Invest* **35**: 199-203, 1993.

RILEY SC, DUPONT E, WALTON JC, LUU-THE V, LABRIE F, PELLETIER G, CHALLIS JR: Immunohistochemical localization of 3 beta-hydroxy-5-ene-steroid dehydrogenase/delta 5----delta 4 isomerase in human placenta and fetal membranes throughout gestation. *J Clin Endocrinol Metab* **75**: 956-61, 1992.

SAKURAI N, MIKI Y, SUZUKI T, WATANABE K, NARITA T, ANDO K, YUNG TM, AOKI D, SASANO H, HANDA H: Systemic distribution and tissue localizations of human 17beta-hydroxysteroid dehydrogenase type 12. *J Steroid Biochem Mol Biol* **99**: 174-81, 2006.

SHAFQAT N, MARSCHALL HU, FILLING C, NORDLING E, WU XQ, BJORK L, THYBERG J, MARTENSSON E, SALIM S, JORNVALL H, OPPERMAN U: Expanded substrate screenings of human and *Drosophila* type 10 17beta-hydroxysteroid dehydrogenases (HSDs) reveal multiple specificities in bile acid and steroid hormone metabolism: characterization of multifunctional 3alpha/7alpha/7beta/17beta/20beta/21-HSD. *Biochem J* **376**: 49-60, 2003.

SHAXTED EJ, HEYES VM, WALKER MP, MAYNARD PV: Umbilical-cord plasma progesterone in term infants delivered by caesarean section. *Br J Obstet Gynaecol* **89**: 73-6, 1982.

SHEEHAN PM: A possible role for progesterone metabolites in human parturition. *Aust N Z J Obstet Gynaecol* **46**: 159-63, 2006.

SHEEHAN PM, RICE GE, MOSES EK, BRENNECKE SP: 5 Beta-dihydroprogesterone and steroid 5 beta-reductase decrease in association with human parturition at term. *Mol Hum Reprod* **11**: 495-501, 2005.

SHIRAIISHI H, ISHIKURA S, MATSUURA K, DEYASHIKI Y, NINOMIYA M, SAKAI S, HARA A: Sequence of the cDNA of a human dihydrodiol dehydrogenase isoform (AKR1C2) and tissue distribution of its mRNA. *Biochem J* **334** (Pt 2): 399-405, 1998.

SIITERI PK: The continuing saga of dehydroepiandrosterone (DHEA). *J Clin Endocrinol Metab* **90**: 3795-6, 2005.

SIRIANNI R, MAYHEW BA, CARR BR, PARKER CR, JR., RAINEY WE: Corticotropin-releasing hormone (CRH) and urocortin act through type 1 CRH receptors to stimulate dehydroepiandrosterone sulfate production in human fetal adrenal cells. *J Clin Endocrinol Metab* **90**: 5393-400, 2005.

SMITH R, MESIANO S, CHAN EC, BROWN S, JAFFE RB: Corticotropin-releasing hormone directly and preferentially stimulates dehydroepiandrosterone sulfate secretion by human fetal adrenal cortical cells. *J Clin Endocrinol Metab* **83**: 2916-20, 1998.

SMITH R, SMITH JI, SHEN X, ENGEL PJ, BOWMAN ME, MCGRATH SA, BISITS AM, MCELDUFF P, GILES WB, SMITH DW: Patterns of plasma corticotropin-releasing hormone, progesterone, estradiol, and estriol change and the onset of human labor. *J Clin Endocrinol Metab* **94**: 2066-74, 2009.

STECKELBROECK S, JIN Y, GOPISHETTY S, OYESANMI B, PENNING TM: Human cytosolic 3alpha-hydroxysteroid dehydrogenases of the aldo-keto reductase superfamily display significant 3beta-hydroxysteroid

dehydrogenase activity: implications for steroid hormone metabolism and action. *J Biol Chem* **279**: 10784-95, 2004.

STOA KF, BESSESEN A: Identification and quantitation of 5 α -pregnanedione in human pregnancy plasma. *J Steroid Biochem* **6**: 21-5, 1975.

TAKEYAMA J, SASANO H, SUZUKI T, IINUMA K, NAGURA H, ANDERSSON S: 17 β -hydroxysteroid dehydrogenase types 1 and 2 in human placenta: an immunohistochemical study with correlation to placental development. *J Clin Endocrinol Metab* **83**: 3710-5, 1998.

TODOROVIC SM, PATHIRATHNA S, BRIMELOW BC, JAGODIC MM, KO SH, JIANG X, NILSSON KR, ZORUMSKI CF, COVEY DF, JEVTOVIC-TODOROVIC V: 5 β -reduced neuroactive steroids are novel voltage-dependent blockers of T-type Ca²⁺ channels in rat sensory neurons in vitro and potent peripheral analgesics in vivo. *Mol Pharmacol* **66**: 1223-35, 2004.

TORN S, NOKELAINEN P, KURKELA R, PULKKA A, MENJIVAR M, GHOSH S, COCA-PRADOS M, PELTOKETO H, ISOMAA V, VIHKO P: Production, purification, and functional analysis of recombinant human and mouse 17 β -hydroxysteroid dehydrogenase type 7. *Biochem Biophys Res Commun* **305**: 37-45, 2003.

TURKMEN S, LOFGREN M, BIRZNIECE V, BACKSTROM T, JOHANSSON IM: Tolerance development to Morris water maze test impairments induced by acute allopregnanolone. *Neuroscience* **139**: 651-9, 2006.

TURNBULL AC, PATTEN PT, FLINT AP, KEIRSE MJ, JEREMY JY, ANDERSON AB: Significant fall in progesterone and rise in oestradiol levels in human peripheral plasma before onset of labour. *Lancet* **1**: 101-3, 1974.

USAMI N, YAMAMOTO T, SHINTANI S, ISHIKURA S, HIGAKI Y, KATAGIRI Y, HARA A: Substrate specificity of human 3(20) α -hydroxysteroid dehydrogenase for neurosteroids and its inhibition by benzodiazepines. *Biol Pharm Bull* **25**: 441-5, 2002.

VU TT, HIRST JJ, STARK M, WRIGHT IM, PALLISER HK, HODYL N, CLIFTON VL: Changes in human placental 5 α -reductase isoenzyme expression with advancing gestation: effects of fetal sex and glucocorticoid exposure. *Reprod Fertil Dev* **21**: 599-607, 2009.

WALSH SW: Progesterone and estradiol production by normal and preeclamptic placentas. *Obstet Gynecol* **71**: 222-6, 1988.

WATANABE H, HIRATO K, HASHINO M, KOSAKI T, KIMURA T, NAKAYAMA T, YANAIHARA T: Effects of DHA-S on placental 3 beta-hydroxysteroid dehydrogenase activity, progesterone and 20 alpha-dihydroprogesterone concentrations in placenta and serum. *Endocrinol Jpn* **37**: 69-77, 1990.

WEAVER CE, LAND MB, PURDY RH, RICHARDS KG, GIBBS TT, FARB DH: Geometry and charge determine pharmacological effects of steroids on N-methyl-D-aspartate receptor-induced Ca(2+) accumulation and cell death. *J Pharmacol Exp Ther* **293**: 747-54, 2000.

YAWNO T, HIRST JJ, CASTILLO-MELENDZ M, WALKER DW: Role of neurosteroids in regulating cell death and proliferation in the late gestation fetal brain. *Neuroscience* **163**: 838-47, 2009.

YOSHIHARA S, MORIMOTO H, OHORI M, YAMADA Y, ABE T, ARISAKA O: A neuroactive steroid, allotetrahydrocorticosterone inhibits sensory nerves activation in guinea-pig airways. *Neurosci Res* **53**: 210-5, 2005.

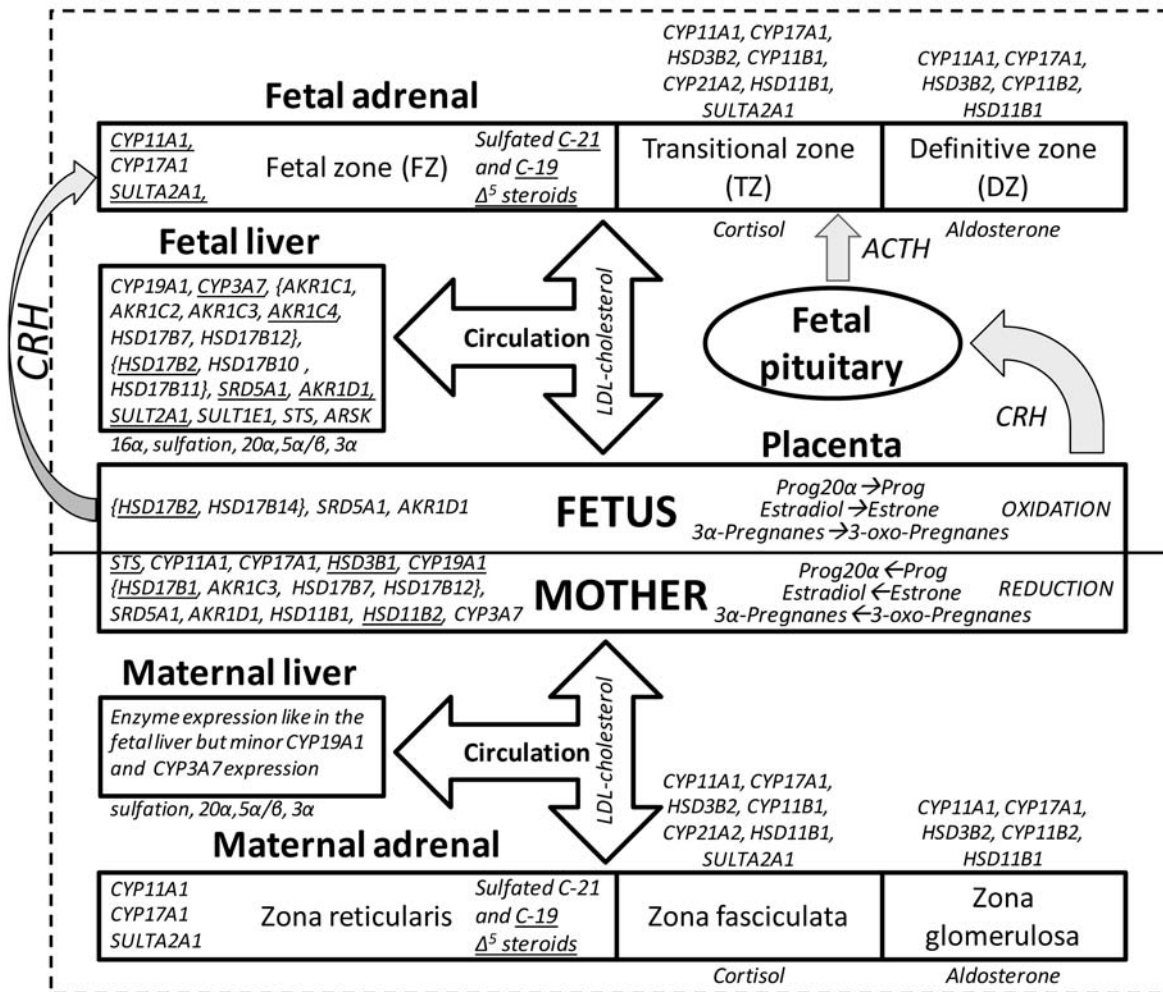


Figure 1. Simplified scheme of steroidogenesis in pregnancy.

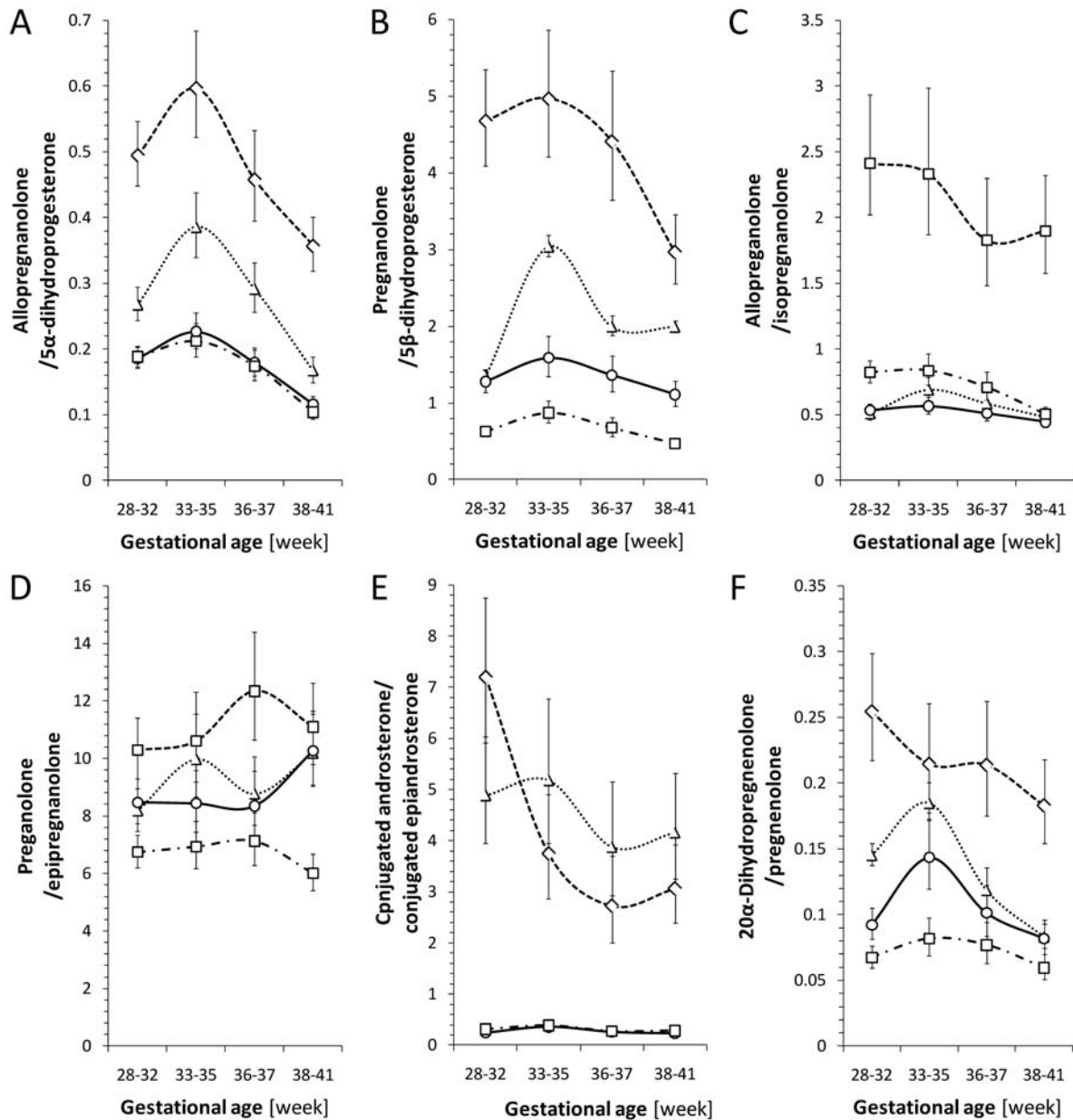


Figure 2: Profiles of ratios of 3 α - to 3-oxo-, 3 α - to 3 β - and 20 α /20-oxo- pregnane and androstane steroids reflecting the balance between inhibiting neuroactive steroids and their inactive and/or antagonistic counterparts in the plasma from the umbilical artery (UA), umbilical vein (UV) and maternal cubital vein (MV) and in amniotic fluid (AF) according to the gestational age (GA, week of gestation). 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 (four body fluids were investigated in each subject), subject factor (separating inter-individual variability), between-subject factor GA (the subjects were separated into 4 groups according to the GA) and body fluid \times GA interaction. Significant body fluid \times GA interaction indicates that there is a significant difference between the dependences of the individual body

fluids on GA. 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 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. The differences between groups according to the body fluid and GA (main factors) for the individual ratios were as follows (only significant ones, $p < 0.05$, are shown): Section A: Body fluid***: AF<MV, AF>UA, AF>UV, MV>UA, MV>UV, GA***: 28-32<33-35, 28-32>38-41, 33-35>36-37, 33-35>38-41, 36-37>38-41, Subject***. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Section B: Body fluid***: AF<MV, AF>UA, AF>UV, MV>UA, MV>UV, UA>UV, GA***: 28-32<33-35, 33-35>36-37, 33-35>38-41, 36-37>38-41, Subject***, Body fluid \times GA **; Section C: Body fluid***: AF<MV, AF<UV, MV>UA, MV>UV, UA<UV, GA***: 28-32>38-41, 33-35>36-37, 33-35>38-41, 36-37>38-41, Subject***; Section D: Body fluid***: AF<MV, AF>UV, MV>UA, MV>UV, UA>UV, Subject***; Section E: Body fluid***: AF>UA, AF>UV, MV>UA, MV>UV, GA*: 28-32>36-37, 28-32>38-41, 33-35>36-37, 33-35>38-41, Subject***, Section F: Body fluid***: AF<MV, AF>UA, AF>UV, MV>UA, MV>UV, UA>UV, GA***: 28-32<33-35, 28-32>38-41, 33-35>36-37, 33-35>38-41, 36-37>38-41, Subject***.

Table 1. Levels of reduced progesterone metabolites in the human body fluids at premature and normal labors from the 28th to 41st week of gestation

Steroid	Our recent data, median (lower quartile; upper quartile) [nmol/l]				Data from other authors (means or medians) [nmol/l]
	Umbilical artery	Umbilical vein	Maternal vein	cubital Amniotic fluid	
5 α -DHP	38.3 (34.6, 53.4)	36.9 (32.9, 54.0)	17.6 (16.0, 25.8)	12.9 (12.4, 13.6)	(GC-MS, w40-42: MV 180 (Stoa and Bessesen 1975); (HPLC-RIA, w36-38: MV 31 (Pearson Murphy <i>et al.</i> 2001); (GC-MS, w36-38 LP: MV 222 (Gilbert Evans <i>et al.</i> 2005); (GC-MS, w40 LP: MV 75.6 (Hill <i>et al.</i> 2007)
P3 α 5 α	4.60 (4.40, 5.60)	3.63 (3.51, 4.47)	6.40 (6.10, 8.20)	2.22 (2.15, 4.91)	(GC-MS, LP: MV 13.5 (Meng <i>et al.</i> 1997); (GC-MS, w38-41 LP: MV 28.8, U 27.0 (Hill <i>et al.</i> 2000); (LC-RIA, VD: MV 157 (Luisi <i>et al.</i> 2000); (GC-MS, w36-38 LP: MV 40.91 (Gilbert Evans <i>et al.</i> 2005); (GC-MS, w40 LP: MV 44 (Hill <i>et al.</i> 2007)
P3 α 5 α C	210 (200, 257)	239 (238, 311)	1087 (1037, 1373)	73.0 (69.0, 114)	(GC-MS, LP: MV 2500 (Meng <i>et al.</i> 1997); (GC-MS, w38-41 LP: MV 358, 141 (Hill <i>et al.</i> 2000); (GC-MS, w40 LP: MV 766 (Hill <i>et al.</i> 2007)
P3 β 5 α	9.50 (9.10, 12.9)	5.96 (5.63, 8.77)	2.87 (2.81, 3.93)	5.99 (5.42, 7.01)	(GC-MS, LP: MV 5.01 (Meng <i>et al.</i> 1997); (GC-MS, w38-41 LP: U 19.9, MV 8.31 (Hill <i>et al.</i> 2000); (GC-MS, w36-38 LP: MV 20.3 (Gilbert Evans <i>et al.</i> 2005); (GC-MS, w40 LP: MV 14 (Hill <i>et al.</i> 2007)
P3 β 5 α C	356 (356, 433)	318 (301, 480)	436 (410, 569)	48.9 (45.7, 75.2)	(GC-MS, w38-41 LP: U 146, MV 169 (Hill <i>et al.</i> 2000); (GC-MS, w40 LP: MV 330 (Hill <i>et al.</i> 2007)
5 β -DHP	9.80 (8.90, 16.2)	9.80 (7.80, 21.2)	1.31 (1.27, 1.78)	1.54 (1.33, 2.38)	(HPLC-RIA, w36-38: MV 2.3 (Pearson Murphy <i>et al.</i> 2001); (HPLC-RIA, VD: MV 178 (Sheehan <i>et al.</i> 2005); (GC-MS, w36-38 LP: MV 3.54 (Gilbert Evans <i>et al.</i> 2005); (GC-MS, w40 LP: MV 4.45 (Hill <i>et al.</i> 2007)
P3 α 5 β	12.8 (12.1, 15.1)	4.42 (3.92, 6.33)	4.40 (4.20, 6.20)	2.87 (2.45, 3.94)	(GC-MS, w38-41 LP: MV 18.9, U 32.8 (Hill <i>et al.</i> 2000); (GC-MS, w36-38 LP: MV 18.51 (Gilbert Evans <i>et al.</i> 2005); (GC-MS, w40 LP: MV 19.7 (Hill <i>et al.</i> 2007)
P3 α 5 β C	184 (177, 253)	193 (175, 229)	475 (444, 573)	108 (101, 208)	(GC-MS, LP: MV 650 (Meng <i>et al.</i> 1997); (GC-MS, w40 LP: MV 435 (Hill <i>et al.</i> 2007)
P3 β 5 β	1.04 (0.89, 1.38)	0.66 (0.60, 1.03)	0.36 (0.33, 0.66)	0.28 (0.17, 0.42)	(GC-MS, w38-41 LP: U 5.92, MV 2.32 (Hill <i>et al.</i> 2000); (HPLC-RIA, w36-38: MV 2.21 (Pearson Murphy <i>et al.</i> 2001); (GC-MS, w40 LP: MV 2.04 (Hill <i>et al.</i> 2007)
P3 β 5 β C	51.5 (47.5, 81.1)	64.6 (59.7, 81.2)	42.9 (40.3, 51.8)	18.4 (16.8, 25.4)	(GC-MS, w38-41 LP: U 14.7, MV 15.8 (Hill <i>et al.</i> 2000); (GC-MS, w40 LP: MV 37.6 (Hill <i>et al.</i> 2007)
P3 α 5 α 20 α	0.382 (0.361, 0.516)	0.233 (0.215, 0.346)	0.423 (0.392, 0.496)	0.276 (0.234, 0.509)	
P3 α 5 α 20 α C	126 (112, 137)	122 (83, 352)	17.5 (14.2, 27.4)	13.6 (11.5, 36.1)	(GC-MS, LP: MV 8900 (Meng <i>et al.</i> 1997)
P3 β 5 α 20 α	2.14 (1.84, 2.65)	1.59 (1.48, 2.35)	1.65 (1.49, 1.95)	0.69 (0.59, 2.14)	
P3 β 5 α 20 α C	1500 (1387, 1947)	1615 (1576, 1750)	4008 (3805, 5412)	530 (365, 956)	(GC-MS, LP: MV 6100 (Meng <i>et al.</i> 1997)
P3 α 5 β 20 α	12.1 (11.6, 15.4)	2.85 (2.57, 3.80)	5.28 (4.34, 8.04)	3.12 (1.98, 7.81)	
P3 α 5 β 20 α C	1657 (1462, 2386)	1362 (1250, 1719)	1806 (1760, 2967)	1090 (916, 1449)	(GC-MS, LP: MV 8100 (Meng <i>et al.</i> 1997)
P3 β 5 β 20 α	0.424 (0.393, 0.569)	0.187 (0.168, 0.268)	0.235 (0.221, 0.335)	0.115 (0.1, 0.274)	

U=mixed umbilical blood, RIA=radioimmunoassay, LP=late pregnancy, w=week of gestation, CS=Caesarean section, VD=vaginal delivery, 5 α -DHP = 5 α -dihydroprogesterone, P3 α 5 α = allopregnanolone, P3 β 5 α = isopregnanolone, 5 β -DHP = 5 β -dihydroprogesterone, P3 α 5 β = pregnanolone, P3 β 5 β = epipregnanolone, P3 α 5 α 20 α = 5 α -pregnane,3 α ,20 α -diol, P3 β 5 α 20 α = 5 α -pregnane,3 β ,20 α -diol, P3 α 5 β 20 α = 5 β -pregnane,3 α ,20 α -diol, P3 β 5 β 20 α = 5 β -pregnane,3 β ,20 α -diol, C=polar conjugates of the steroids