*Physiol. Res. 60: 225-241, 2011*

**REVIEW**

# Reduced Progesterone Metabolites in Human Late Pregnancy

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Received November 20, 2010
Accepted November 25, 2010
On-line November 29, 2010

**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 5α/β-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.

**Key words**

Neuroactive steroids • Pregnancy • Plasma • Metabolome • GC-MS

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## Introduction

Although the effects of neuroactive and neuroprotective 5α/β-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.

## Biosynthesis of neuroactive steroids in human pregnancy

### *Steroid metabolism in fetal and maternal adrenal*

*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) (Fig. 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

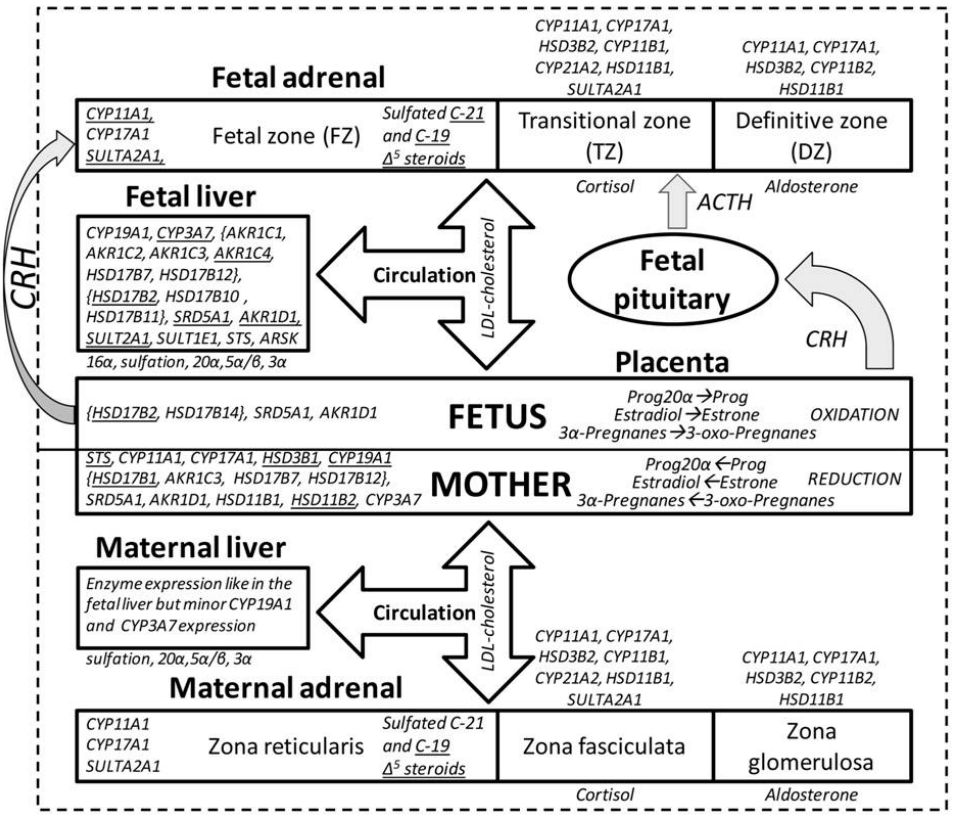


**Fig. 1.** Simplified scheme of steroidogenesis in pregnancy.

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 $\Delta^5$ steroids in the fetal adrenal (Smith *et al.* 1998, Sirianni *et al.* 2005). 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 $\Delta^5$ 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, Moghrabi *et al.* 1997, Leeder *et al.* 2005). The FZ is similar to the adult *zona reticularis* but unlike the adult *zona reticularis*, the FZ produces excessive amounts of sulfated C-21 $\Delta^5$ steroids, including sulfates of pregnenolone and 17-hydroxypregnenolone (Rainey *et al.* 2004, Hill *et al.* 2010a,b). These substances serve as precursors for the placental production of estradiol (Smith *et al.* 1998, Sirianni *et al.* 2005) and progesterone (Jaffe and Ledger 1966, Komatsuzaki *et al.* 1987, Walsh 1988, Hill *et al.* 2010b).

***Steroid metabolism in fetal and maternal liver***

*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 20α-, 17β-, 3α- or 3β-hydroxysteroid dehydrogenase-like activity (Shiraishi *et al.* 1998, Penning *et al.* 2001, Jin *et al.* 2009). 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, 20α-dihydroprogesterone, and GABAergic 3α-hydroxy-5α/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 3α-reduction over the 3β-reduction may catalyze 3α-, 17β-, and 20α-hydroxysteroid

dehydrogenase (HSD) reactions (HSD) (Penning *et al.* 2000, Jin *et al.* 2001, 2009, Usami *et al.* 2002). AKR1C3 catalyzes the reduction of 5α-dihydrotestosterone (5α-DHT), androstenedione, estrone and progesterone to produce 5α-androstan-3α,17β-diol, testosterone, estradiol and 20α-dihydroprogesterone, respectively (Penning *et al.* 2001). AKR1C4, the expression of which is specific for the liver (Nishizawa *et al.* 2000, Penning *et al.* 2000, 2004), catalyzes the transformation of the 5α-DHT into 5α-androstan-3α,17β-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 17β-HSD (HSD17B7), preferring the reduction of the oxo-groups in 20-, 17- and 3-positions to the corresponding 20α-hydroxy-, 17β-hydroxy-, and 3α-hydroxy-counterparts, is also significantly expressed in the liver (Krazeisen *et al.* 1999, Torn *et al.* 2003) as well as type 12 17β-HSD (HSD17B12) catalyzing the transformation of estrone into the estradiol (Sakurai *et al.* 2006).

On the other hand, type 2 17β-HSD (HSD17B2), type 10 17β-HSD (HSD17B10) and type 11 17β-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 20α- and 17β- counterparts (Moghrabi *et al.* 1997). Type 6 17β-HSD (HSD17B6) prefers oxidoreductase and 3(α→β)-hydroxysteroid epimerase activities and acts on both C-19 and C-21 3α-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 5α-androstan-3α,17β-diol to 5α-DHT (He *et al.* 2003) and conversion of 3α-hydroxy-5α-pregnane steroids to the corresponding inactive 3-oxo steroids. The catalysis of HSD17B10 is essential for maintaining normal functions of GABAergic neurons (Shafqat *et al.* 2003). Finally, the HSD17B11 can convert 5α-androstan-3α,17β-diol to androsterone (Brereton *et al.* 2001, Chai *et al.* 2003).

*5α/β-reductases*

Steroid 5α/β reduction is one of the key steps for the biosynthesis of various neuroactive and neuroprotective substances. The liver has high activity of 5α-reductase (SRD5A) and 5β-reductase (AKR1D1) (Meikle *et al.* 1979, Charbonneau and The 2001). Type 1 SRD5A (SRD5A1) is widely distributed in the body, with the highest levels in the liver. SRD5A1 converts testosterone into 5α-dihydrotestosterone and progesterone or corticosterone to their 5α-reduced counterparts. In the peripheral tissues, including the liver, SRD5A1 and reductive 3α-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 5β-reductase (AKR1D1) belonging to AKRs, efficiently catalyzes the reduction of both C-19 and C-21 3-oxo-$\Delta^4$ steroids to the corresponding 5β-reduced metabolites (Kochakian 1983, Okuda and Okuda 1984).

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

***Steroid metabolism in placenta***

*Steroid sulfatases and placental production of sex hormones*

The principal metabolic step that is indispensable for further placental metabolism of sulfated $\Delta^5$ 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) (Ishida *et al.* 1985, Fukuda *et al.* 1986, 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 $\Delta^5$ steroids to the type 1 3β-HSD (HSD3B1) and aromatase(CYP19A1) within the syncytiotrophoblast layer and their conversion to estrogens (Siiteri 2005) and progestogens (Jaffe and Ledger 1966, Komatsuzaki *et al.* 1987, Walsh 1988, Mason *et al.* 1993, Hawes *et al.* 1994, Hill *et al.* 2010a,b).

*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 (Milewich *et al.* 1978, Ishida *et al.* 1985, Fukuda *et al.* 1986) and around parturition (Riley *et al.* 1993, Leslie *et al.* 1994).

*5α/β-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 (Milewich *et al.* 1979, Hill *et al.* 2010a). 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, Sheehan *et al.* 2005, Hill *et al.* 2007).

*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-3β,17β-diol (Peltoketo *et al.* 1999, Lin *et al.* 2006). 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 20α-dihydroprogesterone and conversion of inactive estrone to bioactive estradiol (Peltoketo *et al.* 1999, Penning *et al.* 2001, Li *et al.* 2005, 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 3α-, 17β- and 20α-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 20α-dihydroprogesterone as well as the conversion of bioactive estradiol to biologically inactive estrone (Moghrabi *et al.* 1997). HSD17B2 may also convert the GABAergic 3α-hydroxy-5α/β- C21 steroids to inactive and antagonistic substances but, at the same time, may transform the less active GABAergic 3α,20α-dihydroxy-5α/β-isomers to more active 3α-hydroxy-5α/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.* (1997) 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 17β-HSD (HSD17B14) a member of SDRs may also convert estradiol to estrone and 5-androstene-3β,17β-diol to DHEA (Lukacik *et al.* 2007).

The reversible oxido-reductive interconversion of GABAergic C21 and C19 3α-hydroxy-5α/β-reduced metabolites to the corresponding inactive 3-oxo-metabolites and antagonistic 3β-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 20α-hydroxy-group or a modification of the C17,20 side chain in the 3α-hydroxy-5α/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 5α/β-DHP and 3β-hydroxy-

steroids (isopregnanolone and epipregnanolone), while maternal venous blood contains higher proportions of 20α-hydroxy-steroids like 20α-dihydroprogesterone, 17β-hydroxy-steroids (estradiol) and androstenediol and 3α-hydroxy-5α/β-reduced steroids like GABAergic allopregnanolone and pregnanolone (Fig. 2). Also the levels of conjugated 3α-hydroxy-5α/β-reduced-17-oxo C-19 steroids in MV are pronouncedly higher than in the fetal circulation and amniotic fluid, while the 3β-isomers do not significantly differ (Fig. 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 (Milewich *et al.* 1978, Diaz-Zagoya *et al.* 1979). 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).

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

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

In pregnant women, there are persistently elevated levels of pregnanolone isomers (PI) including the GABAergic 3α-PI (Mickan and Zander 1979, Luisi *et al.* 2000, Pearson Murphy *et al.* 2001, Parizek *et al.* 2005, Hill *et al.* 2007, 2010c, Kancheva *et al.* 2007) (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 5α/β-reduced metabolites rise greatly during pregnancy, being highest for progesterone (562-fold the follicular level), 5α-DHP (161-fold), isopregnanolone (56-fold), allopregnanolone (37-fold), pregnenolone (30-fold), 5β-DHP (16-fold) and epipregnanolone (16-fold) at 37$^{th}$ week of gestation (Luisi *et al.* 2000, Pearson Murphy *et al.* 2001, Parizek *et al.* 2005). 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).

3α- and 3β-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 3α-PI and antagonistic 3β-PI *via* the inactive 3-oxo-intermediate. Sulfation counteracts the effect of 3α-PI on $GABA_A$-r and further amplifies the antagonistic effect of 3β-PI, forming products that negatively modulate $GABA_A$-r on the binding sites, which are different from the sites specific to the unconjugated 3α-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.

### *Changing profiles of the circulating 5α/β-reduced progesterone metabolites during pregnancy*

The increasing trend of PI during pregnancy is more distinct for the GABAergic 3α-PI (Parizek *et al.* 2005) and their polar conjugates increase even more pronouncedly (Hill *et al.* 2007, 2010c). These negative modulators of $GABA_A$-r are present in excessive concentrations in maternal circulation when compared to the free, GABAergic 3α-PI (Park-Chung *et al.* 1999). Rising levels of all conjugated PI and ratios of all conjugates to the respective free steroids during the third trimester indicate increasing liver sulfotransferase activity for all PI (Hill *et al.* 2007, 2010a,c). The ratios of conjugated 5α-PI conjugates to their free counterparts show a gradual increase up to the 37$^{th}$ week, while in the case of 5β-PI these ratios exhibit an accelerating increase from the 31$^{st}$ 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 (Kallela *et al.* 1994, Hering *et al.* 1996). 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 5α/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 $\Delta^5$ steroids or estrogens (Hill *et al.* 2010c).

In the study by Gilbert Evans *et al.* (2005),

allopregnanolone and pregnanolone, as well as progesterone, 5α-DHP, 5β-DHP, isopregnanolone and pregnenolone, in plasma from healthy women increase significantly from 10th to 36th week of pregnancy, while the 5β-DHP and pregnenolone do not change significantly (Gilbert Evans *et al.* 2005). Our previous study demonstrates that the ratio 5α/5β-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 5α/5β-PI ratio near term may indicate decreasing AKR1D1 activity (Parizek *et al.* 2005). The turnovers of 5α-DHP/progesterone and 5β-DHP/progesterone in the third trimester show that the metabolism of progesterone to 5α-DHP inconspicuously culminates in the 35th week, while the conversion of progesterone to 5β-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, Sheehan *et al.* 2005, Sheehan 2006, Hill *et al.* 2010a,c).

The 3α-hydroxysteroid oxidoreductase-mediated turnovers of 5α-DHP and 5β-DHP to allopregnanolone and pregnanolone, respectively, rise during pregnancy, but the turnover of 5α-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 3α/3β-PI during pregnancy (Parizek *et al.* 2005), latter on we have found a mild shift from the 3α-PI to the 3-oxo- and 3β-isomers (Hill *et al.* 2010a). Gilbert Evans *et al.* (2005) show significant but inconsistent changes in PI in the late pregnancy. The authors reported an oxidative shift from allopregnanolone to 5α-DHP but reductive one from 5β-DHP to pregnanolone (Gilbert Evans *et al.* 2005).

***Changes of circulating progestogens and their 5α/β-reduced metabolites around parturition***

Progesterone and 20α-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 and Backström (1990) following the levels of progesterone and 5α-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 5α-DHP, whereas their earlier study reported a significant decrease in 5α-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 5α-DHP were 12 % and 23 % respectively of pre-partum values (Lofgren and Backstrom 1990).

Pearson Murphy *et al.* (2001) demonstrated that during the period 2-7 day postpartum, the level of progesterone fell precipitously, whereas those of pregnenolone and the progesterone metabolites decreased 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,c) 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 3α- to the 3β-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 3β-hydroxy-5α/β-PI to the 3α-isomers are important for the pregnancy sustaining.

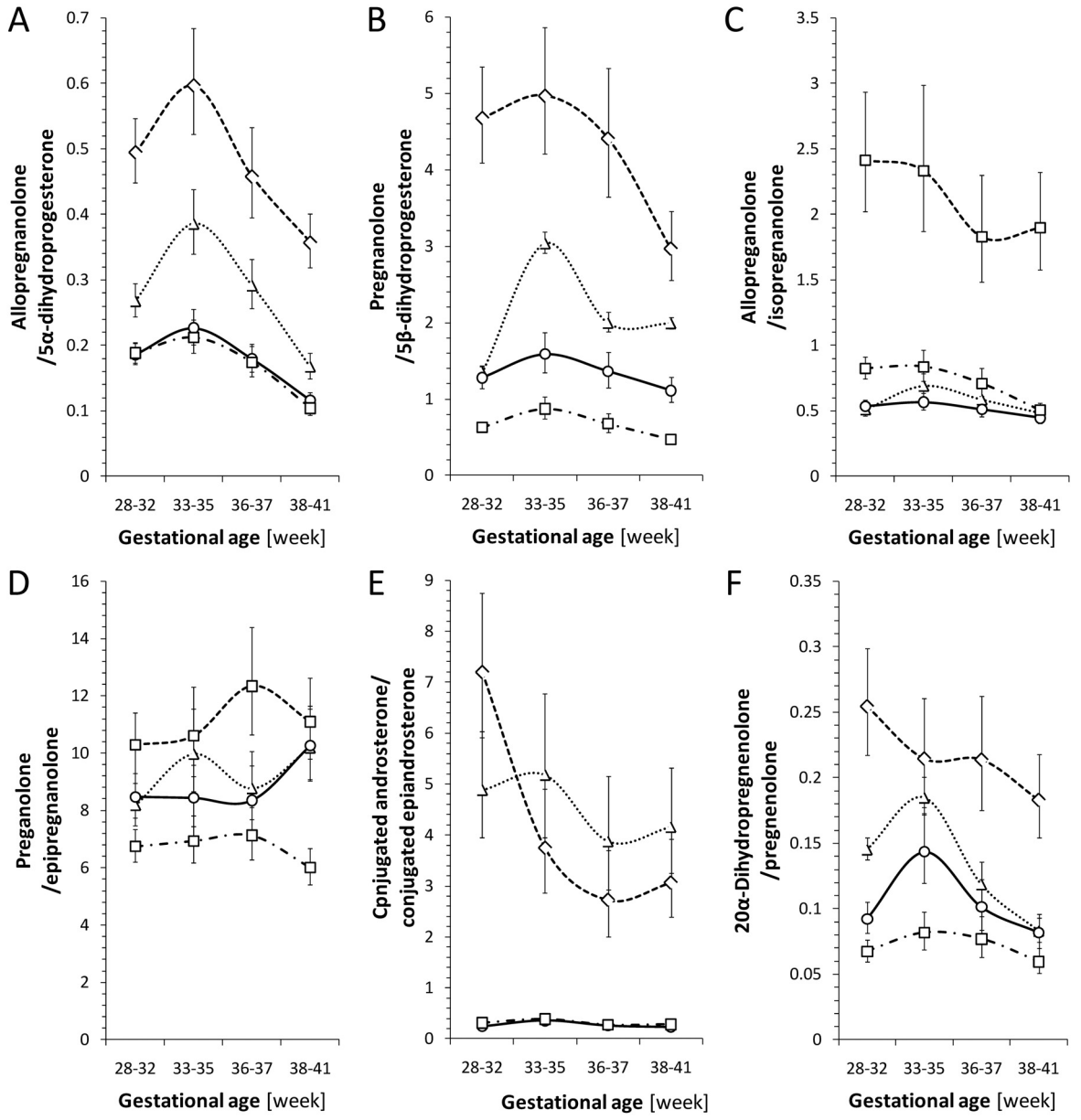


**Fig. 2.** Profiles of ratios of 3α- to 3-oxo-, 3α- to 3β- and 20α/20-oxo- pregnane and androstane steroids reflecting the balance between inhibiting neuroactive steroids and their inactive and/or antagonistic counterparts in the plasma from the umbilical artery (UA), umbilical vein (UV) and maternal 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 × GA interaction. Significant body fluid ×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 × 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 cubital vein | Amniotic fluid | |
| *5α-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 *et al.* 2001); (GC-MS, w36-38 LP: MV 222 (Gilbert Evans *et al.* 2005); (GC-MS, w40 LP: MV 75.6 (Hill *et al.* 2007) |
| *P3α5α* | 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 *et al.* 1997); (GC-MS, w38-41 LP: MV 28.8, U 27.0 (Hill *et al.* 2000); (LC-RIA, VD: MV 157 (Luisi *et al.* 2000); (GC-MS, w36-38 LP: MV 40.91 (Gilbert Evans *et al.* 2005); (GC-MS, w40 LP: MV 44 (Hill *et al.* 2007) |
| *P3α5αC* | 210 (200, 257) | 239 (238, 311) | 1087 (1037, 1373) | 73.0 (69.0, 114) | (GC-MS, LP: MV 2500 (Meng *et al.* 1997); (GC-MS, w38-41 LP: MV 358, 141 (Hill *et al.* 2000); (GC-MS, w40 LP: MV 766 (Hill *et al.* 2007) |
| *P3β5α* | 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 *et al.* 1997); (GC-MS, w38-41 LP: U 19.9, MV 8.31 (Hill *et al.* 2000); (GC-MS, w36-38 LP: MV 20.3 (Gilbert Evans *et al.* 2005); (GC-MS, w40 LP: MV 14 (Hill *et al.* 2007) |
| *P3β5αC* | 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 *et al.* 2000); (GC-MS, w40 LP: MV 330 (Hill *et al.* 2007) |
| *5β-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 *et al.* 2001); (HPLC-RIA, VD: MV 178 (Sheehan *et al.* 2005); (GC-MS, w36-38 LP: MV 3.54 (Gilbert Evans *et al.* 2005); (GC-MS, w40 LP: MV 4.45 (Hill *et al.* 2007) |
| *P3α5β* | 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 *et al.* 2000); (GC-MS, w36-38 LP: MV 18.51 (Gilbert Evans *et al.* 2005); (GC-MS, w40 LP: MV 19.7 (Hill *et al.* 2007) |
| *P3α5βC* | 184 (177, 253) | 193 (175, 229) | 475 (444, 573) | 108 (101, 208) | (GC-MS, LP: MV 650 (Meng *et al.* 1997); (GC-MS, w40 LP: MV 435 (Hill *et al.* 2007) |
| *P3β5β* | 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 *et al.* 2000); (HPLC-RIA, w36-38: MV 2.21 (Pearson Murphy *et al.* 2001); (GC-MS, w40 LP: MV 2.04 (Hill *et al.* 2007) |
| *P3β5βC* | 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 *et al.* 2000); (GC-MS, w40 LP: MV 37.6 (Hill *et al.* 2007) |
| *P3α5α20α* | 0.382 (0.361, 0.516) | 0.233 (0.215, 0.346) | 0.423 (0.392, 0.496) | 0.276 (0.234, 0.509) | |
| *P3α5α20αC* | 126 (112, 137) | 122 (83, 352) | 17.5 (14.2, 27.4) | 13.6 (11.5, 36.1) | (GC-MS, LP: MV 8900 (Meng *et al.* 1997) |
| *P3β5α20α* | 2.14 (1.84, 2.65) | 1.59 (1.48, 2.35) | 1.65 (1.49, 1.95) | 0.69 (0.59, 2.14) | |
| *P3β5α20αC* | 1500 (1387, 1947) | 1615 (1576, 1750) | 4008 (3805, 5412) | 530 (365, 956) | (GC-MS, LP: MV 6100 (Meng *et al.* 1997) |
| *P3α5β20α* | 12.1 (11.6, 15.4) | 2.85 (2.57, 3.80) | 5.28 (4.34, 8.04) | 3.12 (1.98, 7.81) | |
| *P3α5β20αC* | 1657 (1462, 2386) | 1362 (1250, 1719) | 1806 (1760, 2967) | 1090 (916, 1449) | (GC-MS, LP: MV 8100 (Meng *et al.* 1997) |
| *P3β5β20α* | 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, 5α-DHP = 5α-dihydroprogesterone, P3α5α = allopregnanolone, P3β5α = isopregnanolone, 5β-DHP = 5β-dihydroprogesterone, P3α5β = pregnanolone, P3β5β = epipregnanolone, P3α5α20α = 5α-pregnane,3α,20α-diol, P3β5α20α = 5α-pregnane,3β,20α-diol, P3α5β20α = 5β-pregnane,3α,20α-diol, P3β5β20α = 5β-pregnane,3β,20α-diol, C=polar conjugates of the steroids.

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

### *The role of 5α/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.* (1992) reported that 5α-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: $GABA_A$ $\alpha_1$-subunit mRNA expression increased, $\alpha_2$- and δ-subunit mRNA expression decreased during pregnancy, and $\beta_3$-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 $\Delta^5$ steroids and sulfates of 5α-PI (Weaver *et al.* 2000, Malayev *et al.* 2002, Hill *et al.* 2007, 2010a,c). On the other hand, the sulfated 5β-PI, pregnanolone exerts antagonistic effect on the NMDA-r (Park-Chung *et al.* 1997, Weaver *et al.* 2000, Malayev *et al.* 2002) and promotes their desensitization (Kussius *et al.* 2009). The levels of one of the conjugated 5β-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, 2010c).

Catabolizing both 5β-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 5α/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 3α-hydroxy-5α/β-pregnane-steroids/estradiol may be of importance for sustaining the uterine quiescence. Whereas allopregnanolone stagnates from the $36^{th}$ week of gestation (Hill *et al.* 2007), estradiol (Turnbull *et al.* 1974, Buster *et al.* 1979, Parizek *et al.* 2005) 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 5β-position are significantly more potent than the progesterone and 5α-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 > 5β-DHP > epipregnanolone > pregnanolone > progesterone > 5α-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, 5β-reduced metabolites of progesterone may act in pregnancy through a mechanism mediated by pregnane X-type receptors (Putnam *et al.* 1991, Mitchell *et al.* 2005).

The aforementioned effects of 5α/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 5α/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, Sheehan 2006, Hill *et al.* 2007, 2010a,c).

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

*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.

*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, 5β-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.

***Neuroprotective and excitotoxic effects of 5α/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.* (2006) and from the increased stress-induced peripheral production of progesterone.

The data summarized in this review shows that besides the progesterone, a variety of its 5α/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.

## Conflict of Interest

There is no conflict of interest.

## Acknowledgements

This study was supported by grant IGA MZ ČR NS/9834-4. This review is dedicated to our respected tutor, doyen of steroid biochemistry Professor Luboslav Stárka, MD, PhD, DSc on the occasion of his anniversary.

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