

Enzymes involved in the formation and transformation of steroid hormones in the fetal and placental compartments[☆]

J.R. Pasqualini^{*}

Hormones and Cancer Research Unit, Institut de Puériculture et de Périnatalogie, 26 Boulevard Brune, F-75014 Paris, France

Abstract

Human fetal and placental compartments have all the enzymatic systems necessary to produce steroid hormones. However, their activities are different and complementary: the fetus is very active in converting acetate into cholesterol, in transforming pregnanes to androstanes, various hydroxylases, sulfotransferases, whilst all these transformations are absent or very limited in the placenta. This compartment can transform cholesterol to C21-steroids, convert 5-ene to 4-ene steroids, and has a high capacity to aromatize C19 precursors and to hydrolyse sulfates. Steroid hormone receptors are present at an early stage of gestation and are functional for important physiological activities. The production rate of some steroids increases drastically with fetal evolution (e.g. estriol increases 500–1000 times in relation to non-pregnant women). We can hypothesize that the control of active steroid hormones could be carried out by fetal and placental factors, which act by stimulating or inhibiting the enzymes involved in their formation and transformation during pregnancy evolution and, consequently, limiting the high levels of the biologically active hormone.

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1. Introduction

The crucial role of ovarian hormones in supporting pregnancy is largely recognized. The corpora lutea of the cycle are converted into those of pregnancy by a signal that emanates from the fetoplacental unit. The chorionic gonadotropin secreted by the placenta assumes the role of maintaining the corpus luteum and directing placental steroidogenesis. Initially placental gonadotropins are luteotropic, facilitating a continuous secretion of estrogens and progesterone from the corpus luteum. Later in pregnancy, the placenta may assume total steroidogenic potential and the corpus luteum is no longer needed to bring fetal development to term.

The enzyme systems involved in the formation and transformation of steroid hormones evolve with the progress of pregnancy. There are significant differences in quality and quantity of enzymes between the fetal and placental compartments, although they remain complementary. The fetoplacental unit has the capacity to biosynthesize all the active

steroids (e.g. androgens, estrogens, gluco- and mineralocorticosteroids, progestins) which play an important biological role during gestation.

The steroid hormone receptors are present at an early age in the target tissues of the fetus. It is suggested that the hormone–receptor complex may be involved in the program mechanism for normal physiological, or for pathological, conditions in extra-uterine life.

Here we summarize the enzymes involved in the formation and transformation of steroid hormones in the fetoplacental unit, their evolution and their possible biological role.

2. Formation and transformation of steroid hormones in the fetoplacental unit

The major advances in understanding the biosynthesis and metabolism of steroids were carried out with the use of radioactive hormone precursors that made it possible to study hormonal formation and transformation in physiological conditions. Westin et al. [1] and Nyberg and Westin [2] were the pioneers in the use of human fetal perfusion techniques. The

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^{*} Tel.: +33 1 45 39 91 09/42 41 21; fax: +33 1 45 42 61 21.

E-mail address: jorge.pasqualini@wanadoo.fr.

methods that made these studies possible include: in situ placental perfusion; in situ administration of the hormone or its precursors in the fetoplacental unit; simultaneous injection of one tracer into the anticubital vein of the mother and of a different one into the amniotic fluid; and incubation of fetal tissues or isolated fetal cells. These techniques and their applications were extensively developed by E. Diczfalusy and co-workers at the Karolinska Sjukhuset, Stockholm, Sweden (for a review, see [3]).

2.1. Formation and transformation of cholesterol

Fetal perfusion of ^{14}C -acetate allows its conversion into cholesterol, as well as into various 5-ene-steroids such as pregnenolone, pregnenolone sulfate, dehydroepiandrosterone and its sulfate [4,5]. Carr and Simpson [6] hypoth-

esized that cholesterol utilized by the fetal adrenals is the principal source of steroid biosynthesis. These authors also demonstrated that the *de novo* synthesis of cholesterol is stimulated by ACTH [7].

The biosynthesis of cholesterol in the placenta is very limited; the data is substantially confirmed by the lack of conversion of acetate to squalene, lanosterol or cholesterol, following placental perfusion.

The placenta uses the cholesterol received from both the fetal and maternal compartments. It is hydroxylated to 20α , $22R$ -dihydroxycholesterol and, via the effect of desmolase, is converted to pregnenolone. The cytochrome P450 cholesterol side-chain cleavage (CYP11A) and its mRNA are expressed at very high levels in the fetal adrenals, testes and placenta [8]. Fig. 1 gives an overview of the formation of cholesterol in these compartments.

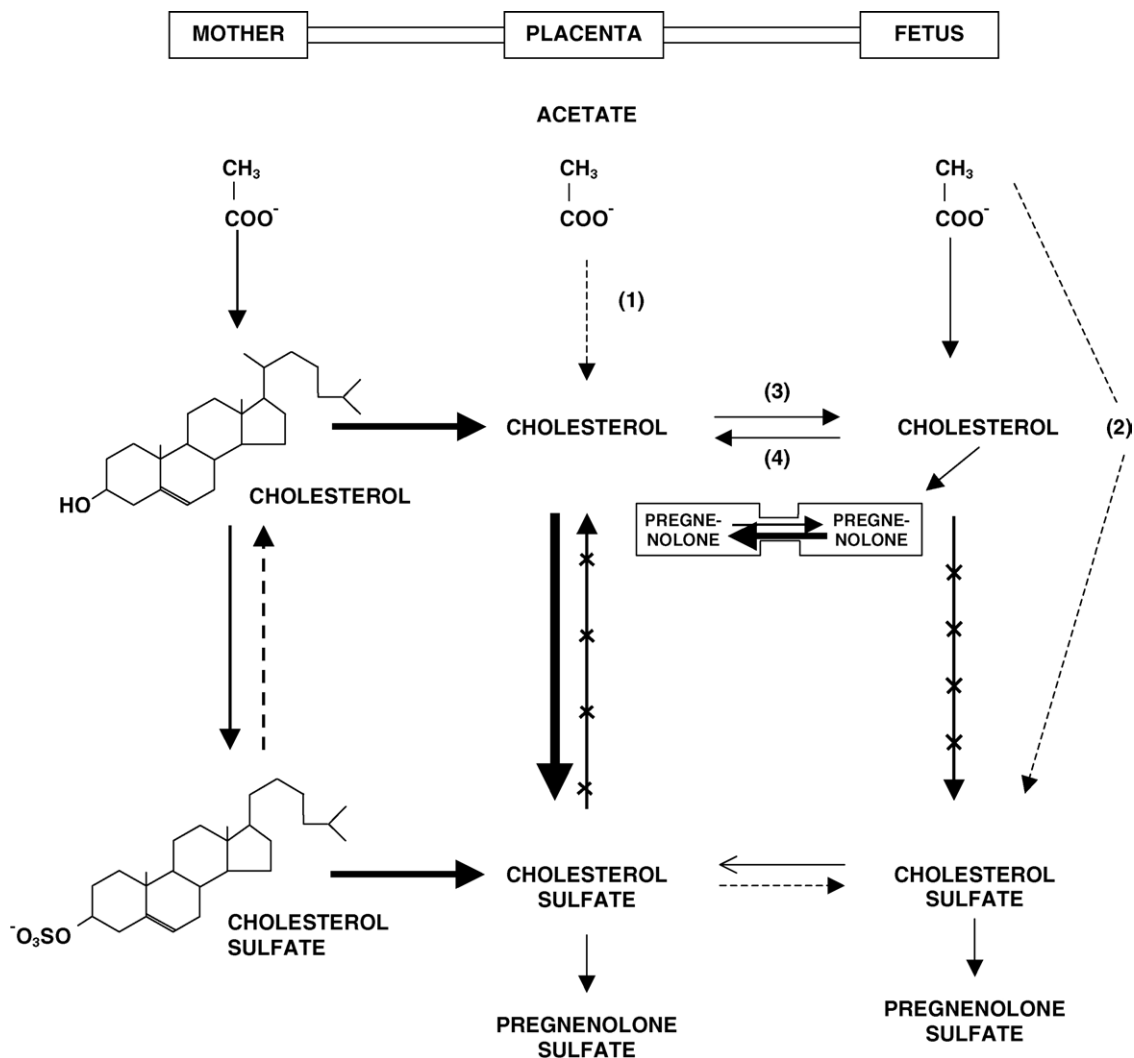


Fig. 1. Cholesterol biosynthesis and transformation in the maternal, placental and fetal compartments. Acetate is the main source of cholesterol in both the maternal and fetal compartments. (1) In the placenta, the conversion is limited. Sulfation takes place readily in the mother, but not in the placenta. In the fetus, perfusion of labeled acetate yields cholesterol sulfate (2) but the ester is not formed following perfusion of cholesterol; the biosynthetic pathway that leads to the ester is not known. The placenta is permeable to cholesterol from the mother (3) but the fetus also secretes cholesterol to the placenta (4). (————→) Major pathways; (-----→) minor contribution; (.....→) postulated pathway; (x-----→) excluded pathways.

2.2. Biosynthesis and transformation of pregnenolone and progesterone

Pregnenolone, via the action of sulfotransferase, is converted into pregnenolone sulfate, particularly in the fetal adrenals. Pregnenolone sulfate can be transformed into 17 α -hydroxypregnenolone sulfate and, via C17–C20 desmolase activity, is converted into dehydroepiandrosterone (DHEA) sulfate. DHEA-sulfate is one of the most abundant steroids present in the fetal organism and is the principal precursor in

the bioformation of placental estrogens. An interesting observation is that, within the first hour and the first day after delivery, the ratio of conjugates:unconjugates of pregnenolone and of pregnenolone isomers decreases very significantly [9]. This could be related to a protective biological role for steroid conjugates (particularly sulfates) during fetal life.

Pregnenolone in the fetal compartment can also be converted to 21-hydroxy-pregnenolone, a “key intermediate” in the biosynthesis of corticosteroids (for details see [3]). In the placenta, pregnenolone is transformed into proges-

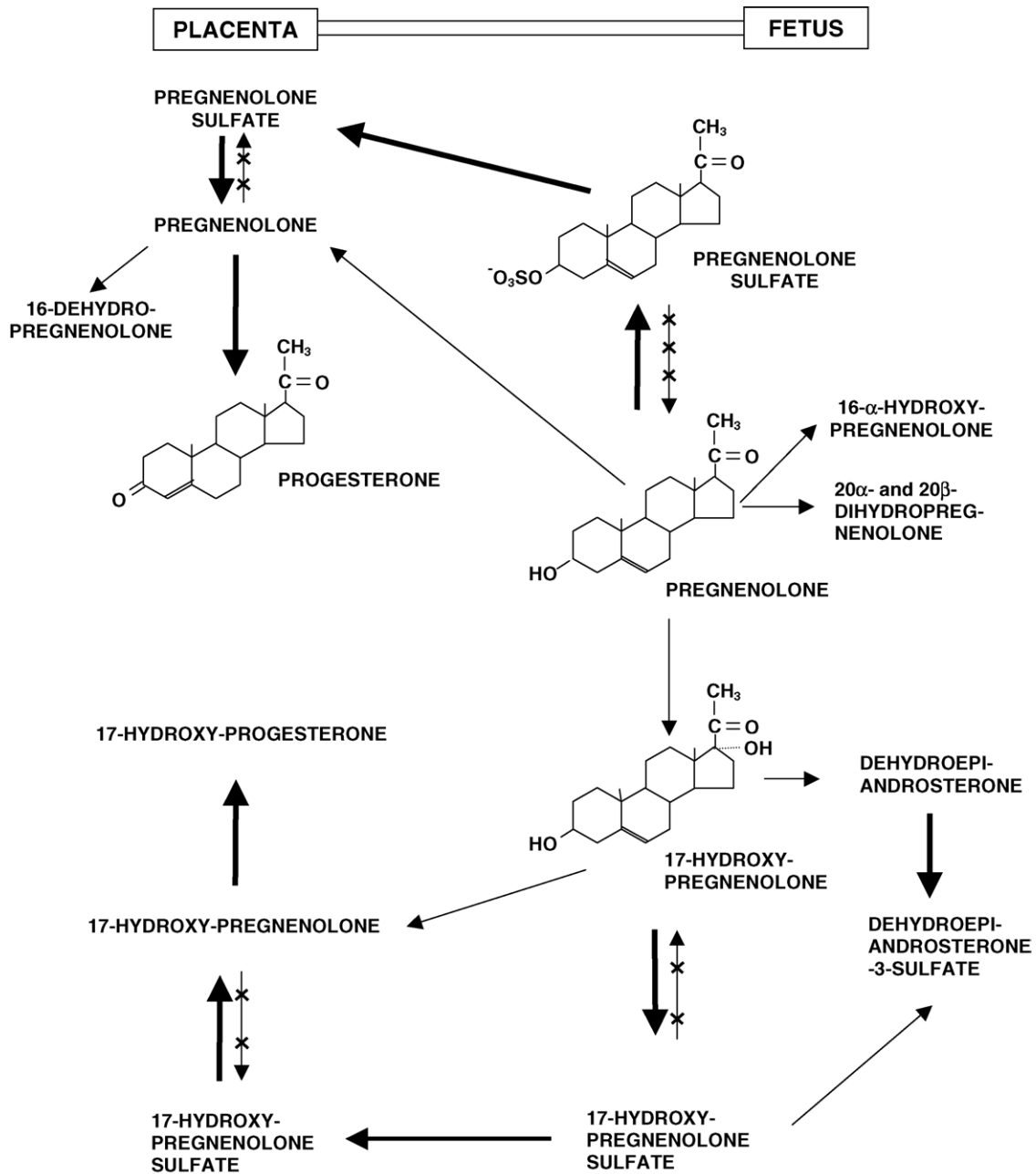


Fig. 2. Metabolism of pregnenolone and 17-hydroxypregnenolone in the fetal and placental compartments. Pregnenolone, 17-hydroxypregnenolone and their sulfates are mainly produced in the fetal compartment. The sulfates are transferred to the placenta, hydrolysed and converted into the respective 3-keto-4-ene derivatives. (→) Major pathways; (→) minor contribution; (---) postulated pathway; (x---x) excluded pathways.

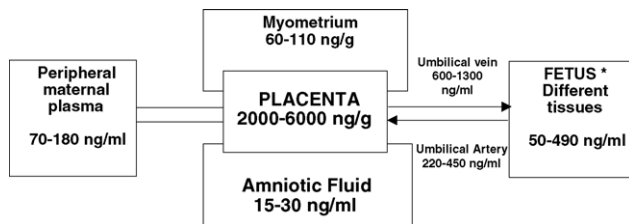


Fig. 3. Progesterone concentration in the different compartments at term. Concentrations in the different tissues were calculated from reports cited in the text. *Fetal concentrations are based on values obtained at 12–18 weeks. The high concentration of progesterone in the placenta is in agreement with the view that this organ is the main source of this hormone.

terone by high 3β -hydroxysteroid dehydrogenase (3β -HSD), 5-ene \Rightarrow 4-ene isomerase activity. 3β -HSD can operate at an early stage of pregnancy, as was demonstrated using a 9-week-old placenta [10]. The rate of conversion of pregnenolone to progesterone is stimulated by human chorionic gonadotropins (hCG) [11]. The main transformation of pregnenolone and 17α -hydroxypregnenolone in the fetal and placental compartments is shown in Fig. 2.

In the third trimester of pregnancy, the production of progesterone reaches 250–350 mg/24 h, of which 80–90% is biosynthesized in the placenta and secreted to both the fetus and the mother. Fig. 3 shows the progesterone concentrations in the various compartments.

Progesterone is largely transformed in both the fetal and placental compartments by the action of reductases, hydroxylases, dehydrogenases, and C17–C20 desmolases. Fig. 4 shows the main metabolic transformation rates of progesterone in these compartments.

2.3. Formation and transformation of gluco- and mineralocorticosteroids

The production of both gluco- and mineralo-corticosteroids by the fetal adrenals is essential to support embryonic functions and later to adapt these to extra-uterine life.

In the human fetus, primitive adrenocortical cells (primordium) appear after 4 weeks of gestation. Two distinct zones are differentiated at 8 weeks: the “inner zone” designated as the “fetal zone” and the “outer zone” that is called the “definitive zone”. The fetal adrenals grow very rapidly; in a 10-week-old fetus, both adrenals weigh about 100 mg, at 20 weeks they weigh around 2 g and at the end of pregnancy about 6–8 g. The fetal zone, which represents 80% of the adrenal weight, disappears a few months after birth and the definitive zone differentiates into the well known three zones: fascicular–reticularis–glomerulosa.

Cortisol synthesis by the fetal adrenals proceeds via two main pathways: the “4-ene pathway”, which utilizes progesterone as a precursor with the following intermediate steps: 17 -hydroxyprogesterone \Rightarrow $17,21$ -dihydroxyprogesterone \Rightarrow cortisol, and the “5-ene-pathway”, which follows the sequence: pregnenolone \Rightarrow 17 -hydroxypregnenolone \Rightarrow $17,21$ -dihydroxypregnenolone \Rightarrow cortisol. The existence

of both pathways was demonstrated in numerous studies employing fetal perfusion and incubation of the whole adrenals (see [3]). The biosynthetic pathways of cortisol are shown in Fig. 5.

What is quantitatively the most important biosynthetic pathway? The high levels of progesterone secreted from the placenta to the fetus, and the marked conversion of progesterone to 17 -hydroxyprogesterone, suggest that the major pathway in cortisol synthesis is through successive hydroxylations of progesterone. This hypothesis was confirmed by various studies using fetal perfusion (see [3]).

Of biological importance is the interconversion of cortisone \Leftrightarrow cortisol by 11β -hydroxysteroid dehydrogenases. In the fetal tissues, as well as in the placenta, the oxidative direction predominates (formation of cortisone) until mid-gestation, but at term, the reverse transformation is in the direction of cortisol formation (11β -hydroxysteroid dehydrogenase type 1). The expression of this enzyme in fetal membranes increases with gestational age. There is also a significant increase in 11β -HSD type 1 expression in the amnion, but not in the chorion, with the onset of labor. It was suggested that the increase of 11β -HSD type 1 expression/activity by intrauterine membranes during late gestation may result in an increased potential for a local increase in cortisol production and that fetal membranes should be considered as an extra-adrenal source for the production of this hormone during late gestation. It has been shown that 11β -HSD type 1 activity is up-regulated by prostaglandins (PG) E2 and F2 α , hormones that are produced in fetal membranes at term [12]. The increase in the local production of cortisol could be related to the mechanism of parturition.

In the fetal compartment, cortisol and cortisone are converted into the corresponding 21-sulfates; only about 5–10% is converted in most tissues, but this is higher in the adrenals (15–25%). Via a very active reductase, these two steroids are transformed in the different fetal tissues into tetra- and hexahydro-derivatives. These transformations are summarized in Fig. 6.

In summary, cortisol is synthesised by the fetal adrenals, mainly in the definitive zone, from an early stage of embryonic development. It is converted into cortisone, which is the main circulating form of this hormone. At the end of pregnancy the situation is reversed and cortisol predominates, which is explained by the important biological role for this hormone during the perinatal period.

Deoxycorticosterone and corticosterone can be biosynthesized from both the 4-ene and 5-ene pathways. These two corticosteroids are largely transformed into the corresponding 21-sulfates in most fetal tissues. This sulfotransferase activity persists for some time after birth. It is notable that corticosterone 21-sulfate can be metabolized into various tetrahydroderivatives without previous hydrolysis (see [3]). In addition, corticosterone can be transformed in the different fetal tissues into 20β -dihydrocorticosterone, 6β -hydroxy-corticosterone and 11β -dehydrocorticosterone (see Fig. 7).

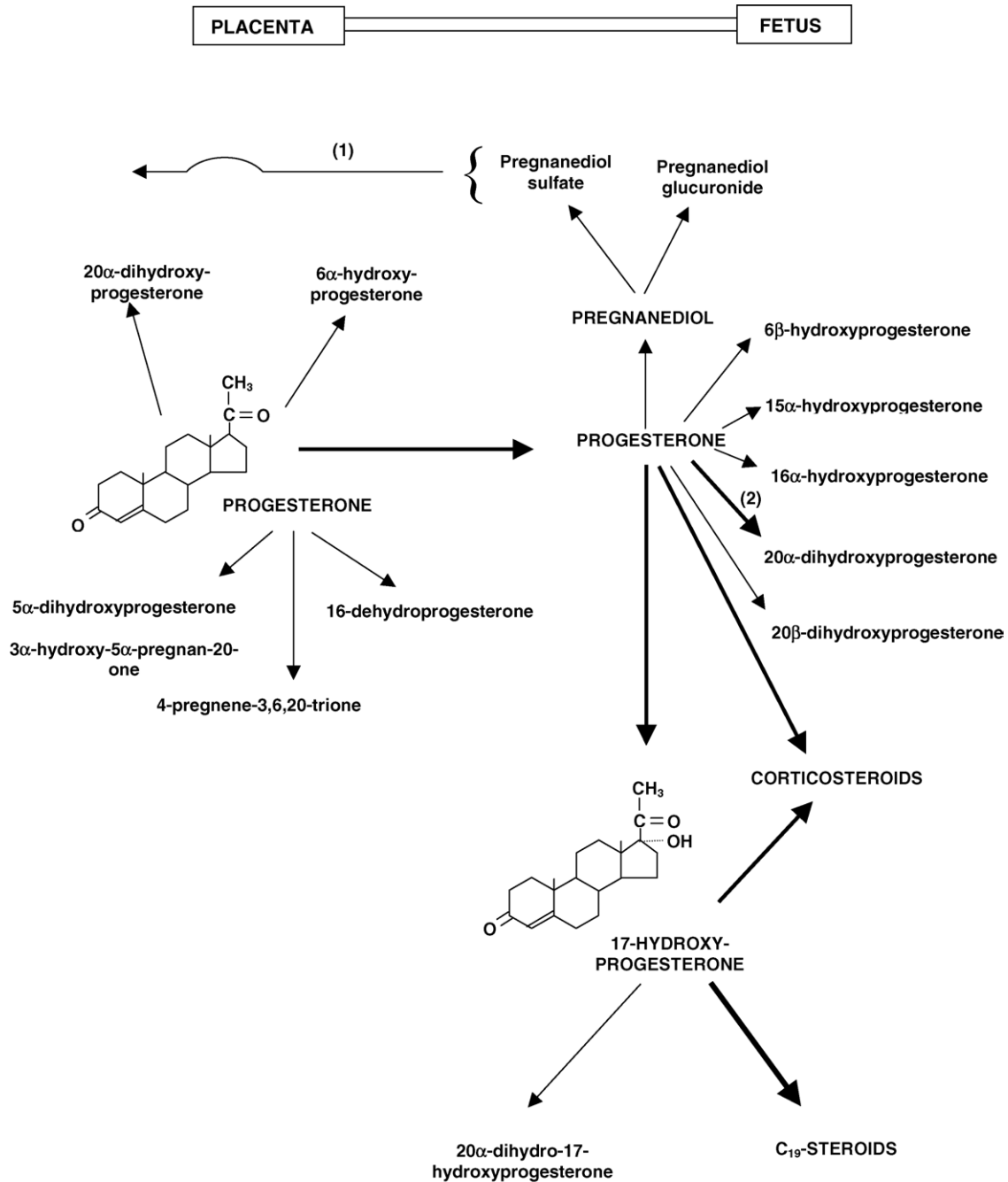


Fig. 4. Main progesterone metabolism in the fetal and placental compartments, fetal progesterone is exclusively of placental origin. (1) Pregnanediol sulfate and pregnanediol glucuronide eliminated in the maternal urine is, in part, of fetal origin. (2) Progesterone in the fetus is reduced mainly to 20α-dihydroprogesterone.

Deoxycorticosterone can be biosynthesized in the placenta: 21-hydroxy-pregnenolone and its sulfate are transferred from the fetal compartment to the placenta, where the sulfate is hydrolyzed and the unconjugated steroid converted by 3β-hydroxysteroid dehydrogenase into high levels of deoxycorticosterone.

Aldosterone, the most potent mineralocorticosteroid, has been demonstrated to be biosynthesized in fetal adrenals at an early stage of gestation. This hormone is largely converted into its tetrahydroderivative, as well as into its 21-sulfate,

mainly in fetal liver (see [3]). These transformations are summarized in Fig. 8.

2.4. Formation and transformation of androgens

The concentration of dehydroepiandrosterone, 16α-hydroxy-dehydroepiandrosterone (16-hydroxy-DHEA) and their corresponding sulfates is higher in the umbilical artery than in the umbilical vein, thus indicating that these androgens are produced mainly in the fetal compartment.

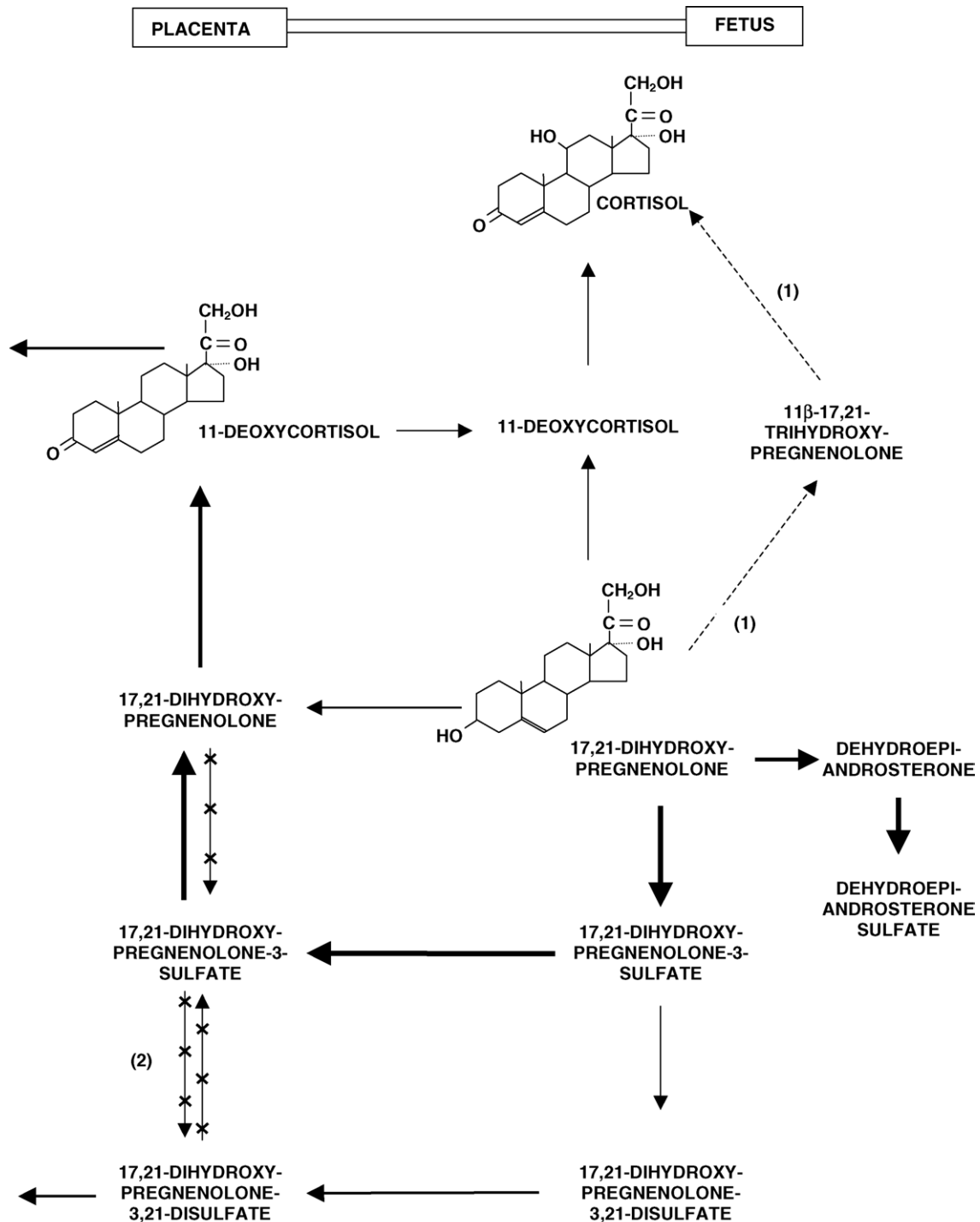


Fig. 5. Formation of cortisol in the fetal and placental compartments. (1) Postulated pathway in the conversion of 17,21-dihydroxypregnenolone to cortisol. (2) Steroid C21-sulfates are hydrolyzed very little or not at all by the placenta.

At term, the total production of DHEA-sulfate reaches 200–220 mg/24 h, of which 90–95% is secreted by the fetus. DHEA is biosynthesized principally in the fetal adrenals and liver from three main precursors: pregnenolone, 17-hydroxypregnenolone and 17,21-dihydroxypregnenolone. The fetal adrenals are very rich in sulfotransferase activity and most of the DHEA produced by this tissue is converted

to the corresponding sulfate. Sulfatase activity for DHEA-sulfate and most steroid sulfates is low or absent in the various fetal tissues. In the fetal compartment, DHEA can be reduced to 5-androstene-3 β ,17(α and β) diols by reductase.

Testosterone is biosynthesized in fetal testes using progesterone and pregnenolone as precursors. Testosterone secretion in the fetus occurs just prior to the onset of male dif-

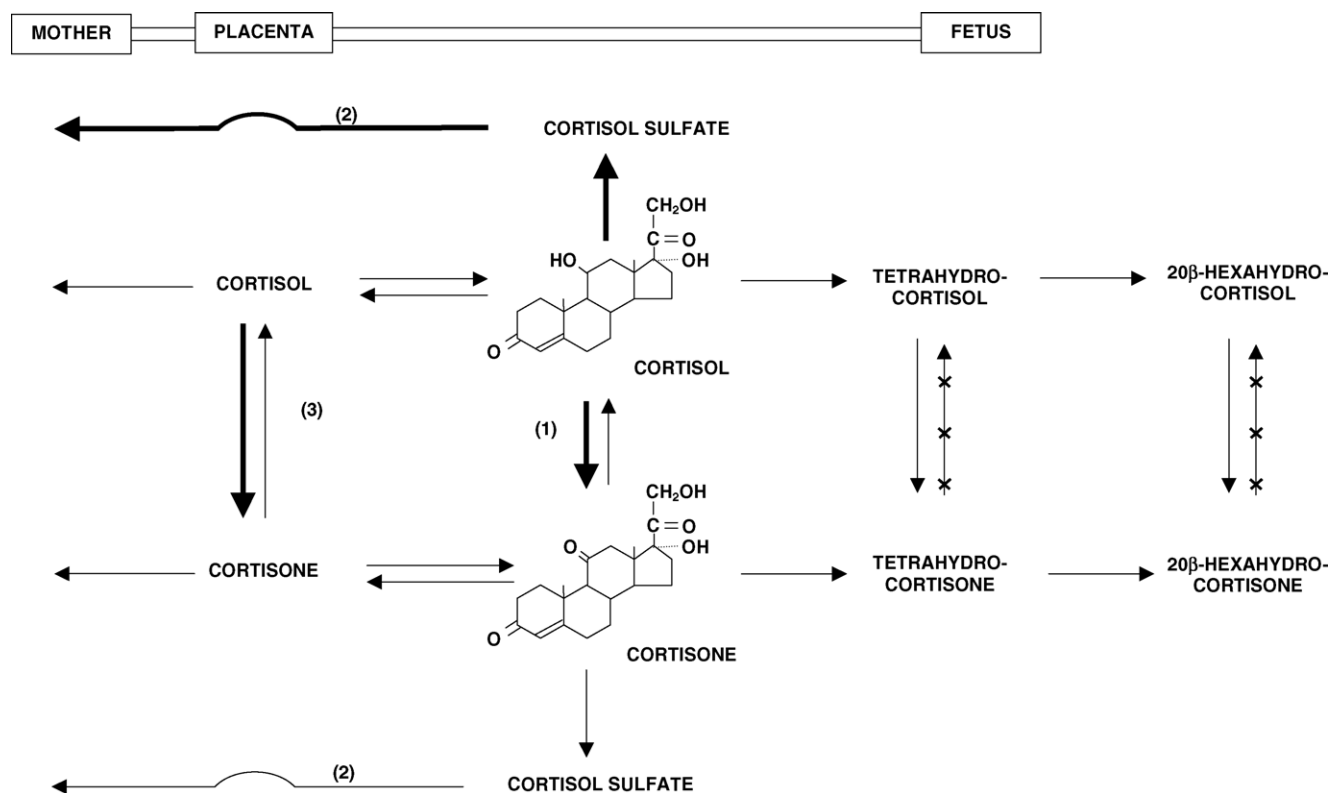


Fig. 6. The metabolism of cortisol and cortisone in the fetal and placental compartments at mid-term. (1) The activity of the 11β -hydroxysteroid-oxydoreductase is very limited in the direction of cortisone \rightarrow cortisol until mid-term; towards term, the direction reverses. (2) Cortisone and cortisol sulfates, metabolites of fetal cortisol, cross the placenta to the maternal compartment without hydrolysis. (3) The conversion of cortisone to cortisol occurs mainly in the chorion.

ferentiation of the urogenital tract (70–90 days gestation). It is converted by 5α -reductase to the potent active androgen 5α -dihydrotestosterone. This compound is formed in significant quantities in the external genitalia, the urogenital tubercle and swelling and the urogenital sinus during the period of fetal sexual differentiation. Fetal liver testosterone is transformed extensively into testosterone sulfate, as well as in etiocholanolone, 5β -androstane- 3α , 17β -diol and their sulfates.

DHEA-sulfate and 16α -hydroxy-DHEA-sulfate are secreted from the fetus to the placenta where they are hydrolyzed by sulfatase. The unconjugated steroids are converted by the activity of 3β -hydroxysteroid dehydrogenase into androstenedione and 16α -hydroxy-androstenedione, respectively, both of which are important precursors of estrogens.

2.5. Formation and transformation of estrogens

Estrogens are formed mainly in the placenta using fetal and maternal precursors. The production rate increases continuously during pregnancy and reaches 100–120 mg/24 h. Estriol, which constitutes 60–70% of the total estrogens, increases 300–500-fold in relation to non-pregnant women. It has been shown that both fetal and maternal precursors are used in about equal proportions in the biosynthesis of

estradiol and estrone, where 90% of the precursors for the formation of estriol are of fetal origin.

In the placenta, the transformation of 5-ene-C19 steroid sulfates to estrogens proceeds by three principal steps:

- (I) Hydrolysis of the sulfates by a sulfatase.
- (II) The 3β -hydroxy-5-ene structure is transformed into a 3-oxo-4-ene moiety by the action of a 3β -hydroxysteroid dehydrogenase, 5-ene \Rightarrow 4-ene isomerase.
- (III) The ring A of the steroid is aromatized in several steps.

Placental capacity for the aromatization of androgens, as well as 17β -hydroxysteroid dehydrogenase activity, increase with the progress of pregnancy. Leuteinizing hormone and hCG have a stimulating action on the aromatization process (see [3]). The expression of human placental aromatase is transcriptionally regulated through the promotor region of exon 1a of the gene. It was suggested that the induction of the aromatase gene in human placenta by cAMP might be mediated by activation of 21-hydroxypregnenolone through a transacting mechanism [13]. The formation of estrone and estradiol by the complementary enzyme activity of the placenta and fetal compartments are summarised in Fig. 9.

Takeyama et al. [14] explored the expression patterns of 17β -HSD isozymes in human fetal tissues; 17β -HSD type 1 was found only in the placenta, whereas 17β -HSD type 2

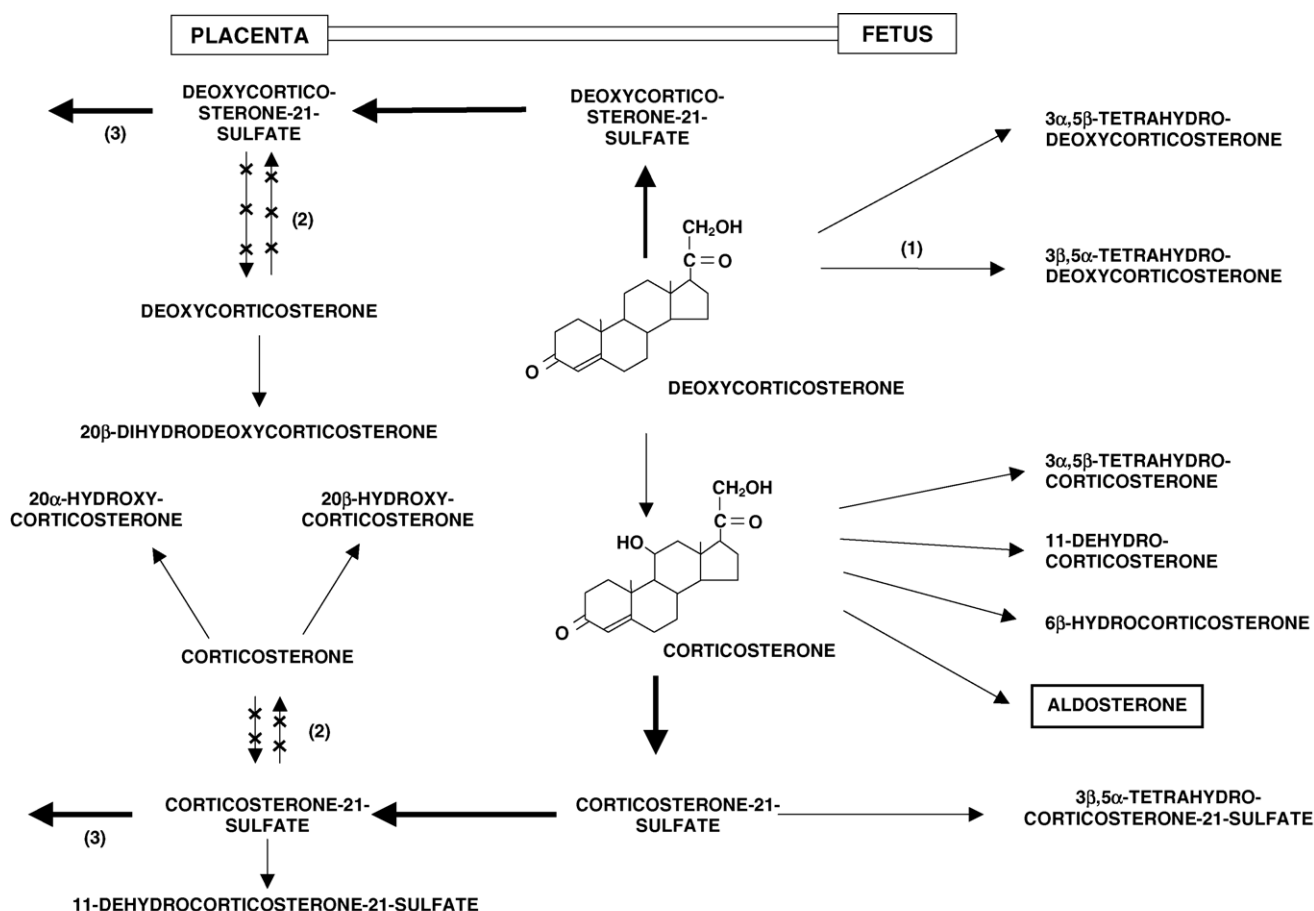


Fig. 7. The metabolism of deoxycorticosterone and corticosterone in the fetal and placental compartments. (1) 3β,5α Tetrahydrocorticosterone is produced exclusively in the fetal compartment. (2) Deoxycorticosterone and corticosterone-21-sulfates are not hydrolysed in the placenta, both are eliminated in the maternal urine without hydrolysis (3).

was detected in the placenta, liver, gastrointestinal tract and urinary tract at 20 weeks gestation. The authors suggest that 17β-HSD type 2 (conversion of estradiol to estrone) may be functioning in the prevention of in utero exposure of the fetus to excessive estradiol from the maternal circulation and amniotic fluids.

The synthesis of estriol in the fetoplacental unit is the culmination of the interrelationship between the fetal adrenal secretion of DHEA-sulfate, transformation to 16α-hydroxy-DHEA-sulfate, mainly in the fetal liver, its transfer to the placenta and aromatization. This sequence – the “neutral pathway” – is the major one in the formation of estriol. The “phenolic pathway” makes only a minor contribution (see Fig. 9). The placenta does not possess 16α-hydroxylase activity and the 16α-hydroxy-C19 precursors must be of either maternal or fetal origin.

The capacity of the fetal tissues for aromatization is limited since most precursors reach the fetus in the form of 3β-hydroxy, 5-ene steroids and the fetal compartment lacks 3β-hydroxysteroid dehydrogenase, 5-ene ⇒ 4-ene isomerase capacity. Estrogens are also hydrolyzed at C15, preferentially

in the fetal compartment, particularly in the fetal liver, and 15α-hydroxyestriol (estretol) is the most abundant derivative formed (see [3]).

Most of the estrogens circulate in fetal compartment as sulfates. The predominant sulfokinase activity is in the C3 position and, as a result, estrone-3-sulfate, estradiol-3-sulfate and estriol-3-sulfate are the most abundant. There is a considerable exchange between the placenta (hydrolysis) and fetal compartment (sulfatation) providing a continuous pool of unconjugated estrogens and estrogen sulfates, which are possibly of great biological significance. The sulfates may act as a “reserve”, yielding active hormones following hydrolysis; the formation of estrogen sulfates may provide a “protective mechanism” since sulfates are biologically inactive.

3. Complementary enzyme activities in the fetal and placental compartments

The fetus lacks certain enzymes that are essential for steroidogenesis (e.g. 3β-hydroxysteroid dehydrogenase, 5-

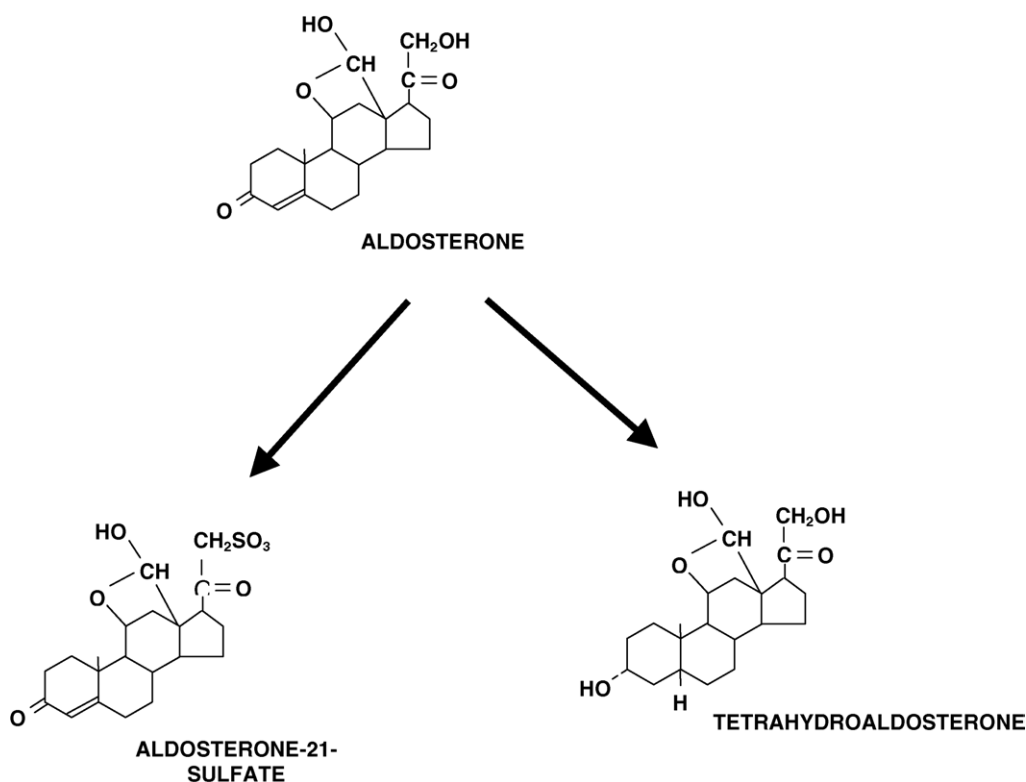


Fig. 8. The metabolism of aldosterone in the fetal compartment.

ene ↔ 4-ene isomerase, aromatase), while other enzymes present in the fetus (e.g. C₁₇–C₂₀ desmolase, hydro-lases) are absent in the placenta. Table 1 summarizes the most important enzymatic functions in the placental and fetal compartments. The fetus is very active in converting acetate into cholesterol and in transforming pregnanes to androstanes by 16-hydroxylation, which is necessary for estriol synthesis. All these transformations are absent in the placenta. This compartment can transform cholesterol to C₂₁-steroids, convert 5-ene to 4-ene steroids, and has a great capacity to aromatize C₁₉ precursors. Thus the fetus and placenta together can elaborate all steroid hormones. Tables 2 and 3 give a general view of the different enzymatic activities in the fetus at mid-gestation and at term in the placenta compared with non-pregnant adult subjects.

Table 1
Predominant enzymatic activities in the placental and fetal compartments in the biosynthesis and transformation of steroid hormones

Placenta	Fetus
1. Sulfatase(s)	1. Sulfokinases
2. 3β-hydroxysteroid dehydrogenase, 5-ene → 4-ene isomerase	2. Cholesterol-synthesizing enzyme
3. Aromatization	3. Different hydroxylases
4. C ₂₀ –C ₂₂ desmolase	4. C ₁₇ –C ₂₀ desmolase
5. 20α,5α-reductases	5. Most reductases

4. Steroid hormone receptors and biological responses in the fetus

The specific binding of different steroid hormones in various fetal organs of humans or animals is well established (for a general review see [15]). At mid-gestation, estrogen receptor (ER)-α was abundant in the fetal uterus and smaller quantities were detected in the ovary, testes, skin and gut. High levels of ER-β-mRNA were present in fetal ovaries, testes, adrenals and spleen. In the uterus, however, ER-α mRNA was more abundant, and ER-β mRNA was expressed only moderately [16]. In another study, it was found that ER-β expression was high in fetal adrenal gland but low levels of this messenger were present in the fetal brain, heart, lung and kidney [17].

An interesting aspect is the possible correlation of steroid hormones with growth factors. For instance, in the isolated uterus of fetal guinea pig, epidemial growth factor (EGF) increased progesterone receptors; however, transforming growth factor (TGF)-α and fibroblast growth factor (FGF) were not able to cause a similar effect. Anti-estrogens, tamoxifen and 4-hydroxytamoxifen, can inhibit the progesterone receptor stimulation by EGF. Since in this model estrogen receptors are very low or undetectable, it is suggested that non-estrogen receptor-mediated pathways may be involved in this response [18]. A similar effect of EGF was observed in isolated vaginal cells [19]. The data indicate the poten-

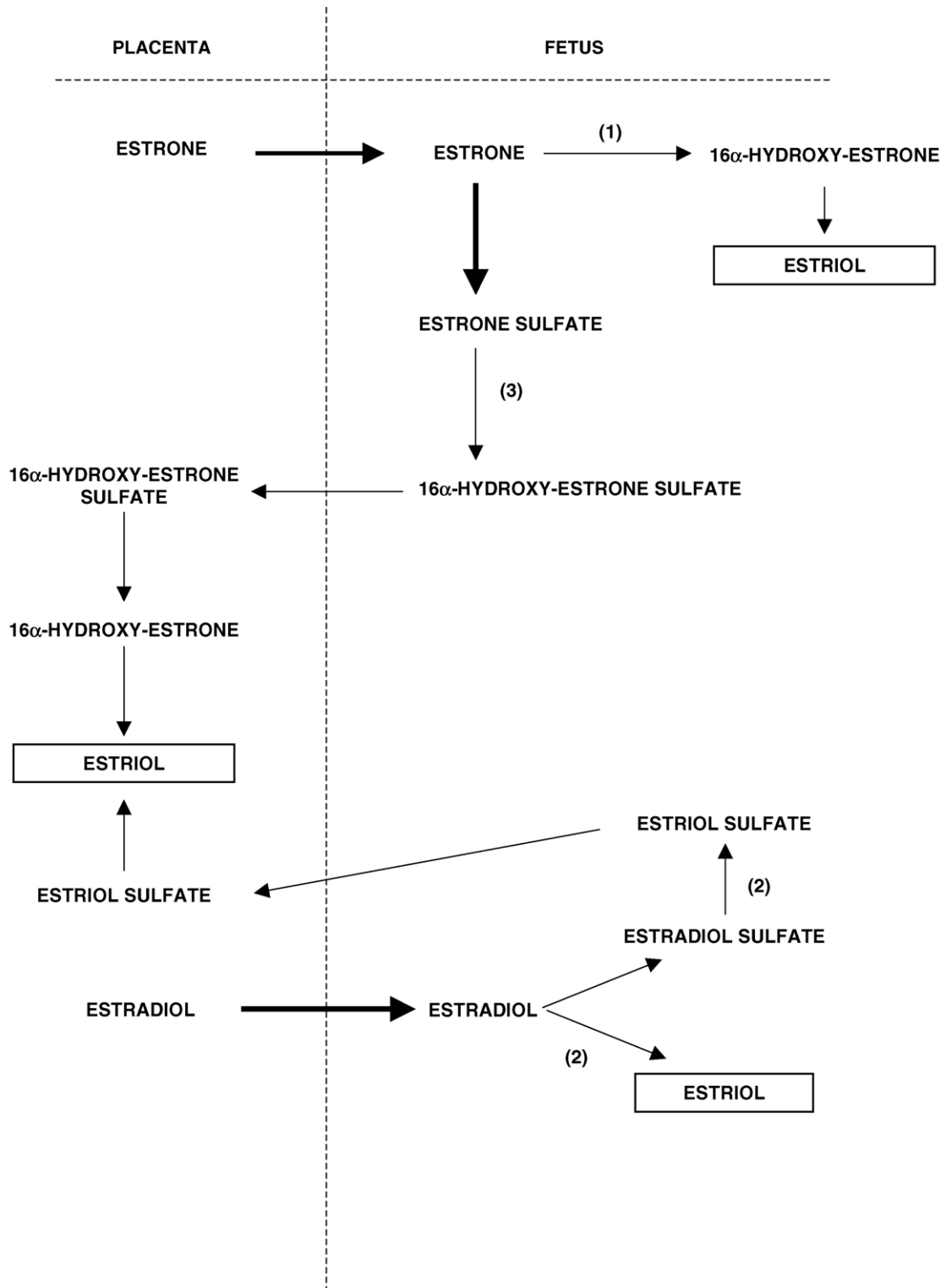


Fig. 9. The 'phenolic pathway' of estradiol synthesis. A minor pathway in estradiol biosynthesis proceeds through 16 α -hydroxylation of estrone (1) or by direct 16 α -hydroxylation of estradiol. 16 α -Hydroxylation of the sulfates of oestrone or oestradiol can also take place (3). The "natural pathway" (see text) is the major one in the bioformation of estradiol.

tial importance of growth factors acting in combination with estrogens during fetal life.

Another attractive aspect of the action of estrogens during fetal life concerns the acetylation of histones. It is very well

known that histones and the high mobility group (HMG) proteins involve conformational changes in chromatin that lead to increased DNA template activity and transcription [20]. The acetylation of these proteins can facilitate the transcrip-

Table 2
Enzymatic activities in the fetus at mid- and term pregnancy, in the placenta and in adults

Enzymatic systems	Mid-gestation	Term	Placenta	Adults	
2-Hydroxylase	+	+	±	+	
4-Hydroxylase	+	+	-	±	
6 α -Hydroxylase	+	+	+	+	
6 β -Hydroxylase	+	+	+	+	
7-Hydroxylase	+	+	-	±	
11 β -Hydroxylase	+++	+++	-	+++	
14-Hydroxylase	+	+	-	+	
15 α -Hydroxylase	+	++	?	±	
16 α -Hydroxylase	++	+++	-	+	
17 α -Hydroxylase	++	++	-	++	
18-Hydroxylase	++	++	-	++	
19-Hydroxylase	±	±	+++	++	
21-Hydroxylase	++	++	-	++	
C ₂₀ -C ₂₂ Desmolase	+	+	+	++	
C ₁₇ -C ₂₀ Desmolase (for steroids with the structure)	$\left\{ \begin{array}{l} 4\text{-ene-C}_{21}\text{-17-OH} \\ 5\text{-ene-C}_{21}\text{-17-OH} \end{array} \right.$	+	+	-	++
		+	+	-	++
11 β -Hydroxysteroid oxidoreductase Transformation 11 β -OH \rightarrow 11-keto Transformation 11-keto \rightarrow 11 β -OH		++	+	+	++
		-	+	+	++
17 β -Hydroxysteroid oxidoreductase (for steroids with the structure)	$\left\{ \begin{array}{l} 5\text{-ene-C}_{19}\text{-17}\beta\text{-OH} \\ 4\text{-ene-C}_{19}\text{-17}\beta\text{-OH} \\ \text{C}_{18}\text{-17}\beta\text{-OH} \end{array} \right.$	+	+	±	++
		+	+	±	++
		+	+	++	++
20-Hydroxysteroid oxidoreductase Transformation	20-keto \rightarrow 20 α -OH	++	++	+	+
	20-keto \rightarrow 20 β -OH	+	+	±	+
	20 α -OH \rightarrow 20-keto	+	+	++	+
	20 β -OH \rightarrow 20-keto	+	+	-	+
3 β -Hydroxysteroid dehydrogenase 5-ene 4-ene isomerase (for steroids with the structure)	$\left\{ \begin{array}{l} 5\text{-ene C}_{19} \\ 5\text{-ene 21 CH}_3 \\ 5\text{-ene 21 CH}_2\text{OH} \\ 5\text{-ene 17 OH,21-CH}_2\text{OH} \end{array} \right.$	±	±	+++	++
		-	±	+++	++
		+	+	+++	++
		+	+	+++	++
Aromatisation of Ring A		±	±	+++	++
Reductase in Ring A	$\left\{ \begin{array}{l} 3\alpha,5\beta\text{-Tetrahydro derivative} \\ 3\alpha,5\alpha\text{-Tetrahydro derivative} \\ 3\beta,5\alpha\text{-Tetrahydro derivative} \\ 5\alpha\text{-Dihydro derivative} \\ 5\beta\text{-Dihydro derivative} \end{array} \right.$	+	+	?	+++
		+	+	?	++
		+	+	?	±
		+	+	+	++
		+	+	+	++

(±) Weak enzymatic activity; (+) moderate; (++) intense; (+++) very intense; (?) not known or not studied; (-) absence of enzyme activity. (*) Intense transformation of cortisone (11-keto) into cortisol (11 β -OH) is found in the chorion laeve.

Table 3

Relative activities of sulfokinases, glucuronyltransferases, sulfatases and β -glucuronidases in the fetus at mid- and term pregnancy, in the placenta and in adults

Enzymatic systems	Steroid	Structure	Mid-gestation	Term	Placenta	Adults
Sulfokinases for steroids with the structure	C ₁₈	OH in C ₃	+++	+++	–	++
		OH in C ₁₆ (α)	±	±	–	±
		OH in C ₁₇ (β)	±	±	–	±
	C ₁₉	4-ene, OH in C ₁₇ (β or α)	+	+	–	±
		5-ene, OH in C ₃ (β)	+++	+++	–	++
	C ₂₁	4-ene, OH in C ₂₁	+	++	–	+
5-ene, OH in C ₃ (β)		+++	+++	–	+	
Sulfatase for steroids with the structure	C ₁₈	SO ₃ [–] in C ₃	–	–	+++	+
		SO ₃ [–] in C ₁₆	–	–	?	?
		SO ₃ [–] in C ₁₇	–	–	?	?
	C ₁₉	3 α -SO ₃ [–]	–	–	±	+
		5-ene, 3 β -SO ₃ [–]	–	–	+++	+
		17 β -SO ₃	–	–	±	±
C ₂₁	5-ene, 3 β -SO ₃	–	–	+++	+	
	SO ₃ in C ₂₁	–	–	±	+	
β -Glucuronidase for gluconurides of C ₁₈ , C ₁₉ or C ₂₁ steroids			–	?	–	+
Glucuronyl transferase for steroids with the structure	C ₁₈	OH in C ₃	±	±	–	+
		OH in C ₁₆ (α)	+	+	–	++
		OH in C ₁₇ (β)	?	?	–	±
	C ₁₉	OH in C ₃ (α)	±	?	–	++
		5-ene, OH in C ₃ (β)	–	–	–	±
	C ₂₁	OH in C ₃ (α)	±	±	–	++
		OH in C ₃ (β)	?	?	–	±
		OH in C ₂₁	?	?	–	±

For explanation of symbols see Table 2.

tional process. Estradiol injected directly into the fetus of guinea pigs can stimulate 7–10-fold the acetylation of nuclear histones H2, H3, and H4 in the fetal uterus [21]. Also, with this treatment, the acetylation of HMG proteins 1 + 2 are stimulated by 33% and HMG 14 + 17 by 130% [22]. This effect of estradiol during fetal life is of vital importance as it has been established that acetylated histones are involved as co-activators of ERs [23,24].

Respiratory distress syndrome (RDS) is one of the major causes of neonatal morbidity and mortality. RDS is caused by an inability of the neonatal lung to produce adequate quantities of surfactant, a lipoprotein substance that reduces surface tension within the lung alveolus and thereby prevents alveolar collapse upon exhalation of air. It has been found that surfactant glycerophospholipid and protein synthesis in fetal lung tissue is regulated by glucocorticosteroids, thyroid hormones, prolactin, estrogens, androgens, catecholamines acting through β -adrenergic receptors and cyclic AMP, growth factors, cytokines and insulin [25]. All of these examples show the biological importance of steroid hormones during fetal evolution.

5. The fetal enzyme hypothesis: its possible role in breast cancer

As was indicated in the previous section, most of the estrogens (90–95%) in the fetal compartment circulate in the form of sulfate conjugates. These conjugates are biologically

inactive and must be hydrolyzed by sulfatase before eliciting a biological response. Hence, the control over this enzyme during fetal life is of prime importance in regulating the action of estrogen. In a series of studies in our laboratory using human breast cancer cell lines it was shown that progesterone and various progestins, as well as tibolone and its metabolites, are potent anti-sulfatase agents (for a recent review see [26]). It was also found that estradiol in breast cancer cells can decrease its own bioformation by blocking estrone sulfatase activity [27]. Provided that this paradoxical effect of estradiol works in the fetoplacental unit, it can be another effective way to control the response of the hormone during fetal evolution.

Estrogens also originate from the conversion of androgens by the action of aromatase. Because these two enzymes, sulfatase and aromatase, are localized mainly in the placental compartment, it is suggested that their control may operate principally in this tissue.

Other important enzymes to consider in the fetal and placental compartments are those involved in the conversion of the weak estrogen, estrone, to the bioactive estradiol through the action of 17 β -HSD type 1 and in reverse the estradiol to estrone by 17 β -HSD type 2. It was also demonstrated that progesterone and progestins can control these interconversions in breast cancer cells [27]. The sulfotransferase activity, localized mainly in the fetal compartment, can play an important role in the metabolism of these hormones during fetal life by converting unconjugated estrogens to the biologically inactive sulfates; consequently, its control by stimulatory fac-

tor(s) (e.g. progesterone and progestins) [27] can block the hormonal response.

The biological action of estrogens can be effected by other metabolic products including those with hydroxylations in the C₂, C₄, and C₁₆ positions. The conversion of estrone and estradiol to C₂-hydroxy derivatives in breast cancer tissues, and the subsequent formation of catechol *O*-methyl estrogen by the action of *O*-methyl-transferase, is well documented [28,29]. It is interesting that 2-methoxy estradiol can inhibit the proliferation of breast cancer cells [30]. As this anti-proliferative effect can be obtained in negative ER cell lines, it is suggested that the biological response of 2-methoxy estradiol is mediated by another pathway than the classical ER. This assumption is confirmed by the fact that the binding affinity to ER is only 0.1% of that seen with estradiol [31]. It was suggested that 2-methoxy-estradiol can have anti-tumorigenic and anti-angiogenic effects that can protect from estrogen-induced cancer in target organs [32].

On the other hand, 4-hydroxy estrone and 4-hydroxy estradiol can be considered as carcinogens because they can

provoke kidney cancer, as shown after administration to the male Syrian hamster [33]. These derivatives can also exert a stimulatory effect on the growth of breast cancer cells [34]. 16 α -Hydroxy estrone was also postulated to be a tumorigenic agent in humans and in various animal models [35]. It was shown in MCF-7 breast cancer cells that this derivative is capable of accelerating cell cycle kinetics and stimulating the expression of cell cycle regulatory proteins [36]. Fig. 10 schematizes these metabolic transformations of estrone and estradiol.

What might be the impact of these metabolic transformations of estrogens in fetal breast cells? Could the “intracrine concept”, where a hormone is biosynthesized in the same organ where its biological response is carried out, apply to fetal breast tissue? How is the inactivation of a hormone by binding to specific plasma or cellular proteins effected? Finally, how is the activity controlled of different enzymes (sulfatase, aromatase, 17 β -HSD, sulfotransferases, hydroxylases) that contribute to estrogen biosynthesis and transformation in the fetal and placental compartments through fetal

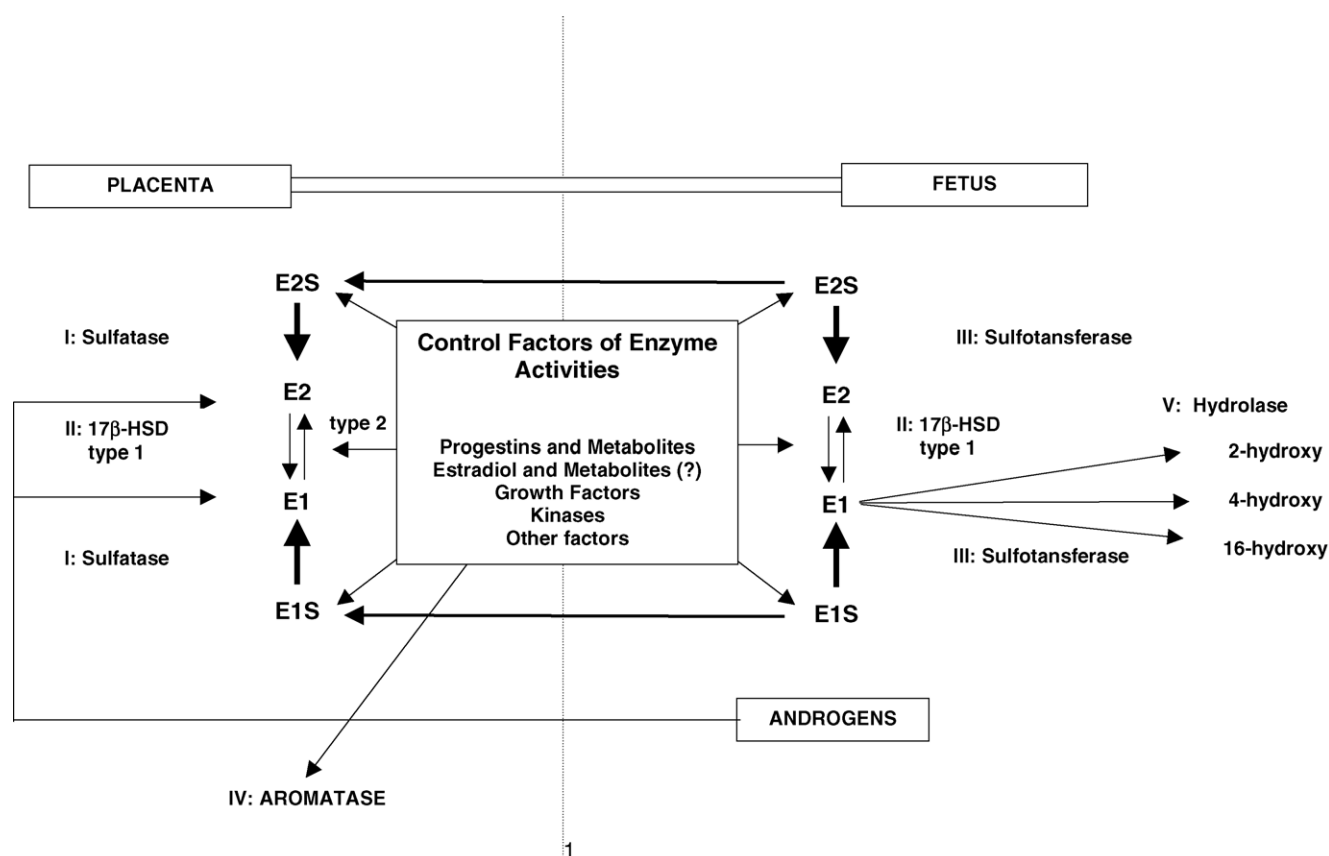


Fig. 10. Hypothetical control of the enzymes involved in estrogen formation and transformation in the fetal and placental compartments. Sulfatase (I) and aromatase (IV) activities are very high in the placental compartment and sulfotransferase (III) in the fetal compartment. 17 β -Hydroxysteroid dehydrogenase (II) types 1 and 2 can operate in both the fetal and placental compartments. After Northern blot analysis, the mRNA of 17 β -hydroxysteroid dehydrogenase type 1 was detected only in the placenta, whereas 17 β -hydroxysteroid dehydrogenase type 2 was found in both placental and fetal tissues. However, in RT-PCR analysis, mRNA of 17 β -hydroxysteroid dehydrogenase type 1 was expressed in the placenta, brain, heart, lung and adrenals, but 17 β -hydroxysteroid dehydrogenase type 2 was found in fetal liver, gastrointestinal tract and kidney [14]. Hydroxylases (V) in the C₂, C₄ and C₁₆ position are important metabolic transformation pathways; their role and possible impact on future evolution of the breast (normal and pathological) needs to be explored. The various factors controlling the enzyme activities in both the fetal and placental compartments are of leading importance in regulating the bioactivity of hormones.

evolution and consequently control the biological response of the hormone? These are important aspects to be considered and clarified.

We can hypothesize that the very high levels of progesterone and its metabolites circulating in these compartments, the various steroids or other suppressors or stimulatory factors, play an important role in controlling the enzymes involved in the formation and transformation of estrogens and consequently regulate the future normal or pathological evolution of the target fetal cells [37].

6. Conclusions

Fetal hormonal biosynthetic potential appears early during gestation. With regard to steroid hormones, the adrenal cortex is capable of producing mineralo- and glucocortico-steroids, as well as DHEA, DHEA-sulfate, 16 α -hydroxy-DHEA and its sulfate, and the testes testosterone. This steroidogenesis is regulated by the hypothalamic-pituitary axis. The placenta produces progesterone and estrogens. All the information indicates that there is complementary activity between the enzymes involved in steroid formation and transformation between the placental and fetal compartments: the fetus is very active in converting acetate into cholesterol, in transforming pregnanes into androstanes and in 16 α -hydroxylation, which is necessary for estriol synthesis. All these transformations are absent in the placenta. This compartment can transform cholesterol to C21-steroids, convert 5-ene to 4-ene steroids and has a great capacity to aromatize C19 precursors (see Table 1).

The presence of steroid hormone receptors in various fetal tissues is well documented, as is their correlation with biological responses. Another important and intriguing question is the enormous production of some steroids (e.g. estrogens, progesterone). It was proposed that high concentrations of estrogens in pregnancy increase the probability of breast cancer risk in female offspring by creating a “fertile soil”. We can hypothesize that control of active estrogens and other hormones could be carried out by fetal and placental factors, which act by stimulating or inhibiting the enzymes involved in formation and transformation during pregnancy evolution and, consequently, the high levels of the biologically active hormone.

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