

REVIEW

This paper is dedicated to the 70th anniversary of the founding of Physiologia Bohemoslovaca (currently Physiological Research)

Steroids, Steroid Associated Substances, and Gestational Diabetes Mellitus

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Summary

As gestational diabetes mellitus (GDM) is both a frequent and serious complication, steroid levels in pregnancy are extremely elevated and their role in pregnancy is crucial, this review focuses on the role of steroids and related substances in the GDM pathophysiology. Low SHBG levels are associated with insulin resistance and hyperinsulinemia, while also predicting a predisposition to GDM. Other relevant agents are placental hormones such as kisspeptin and CRH, playing also an important role beyond pregnancy, but which are synthesized here in smaller amounts in the hypothalamus. These hormones affect both the course of pregnancy as well as the synthesis of pregnancy steroids and may also be involved in the GDM pathophysiology. Steroids, whose biosynthesis is mainly provided by the fetal adrenal glands, placenta, maternal adrenal glands, and both maternal and fetal livers, are also synthesized in limited amounts directly in the pancreas and may influence the development of GDM. These substances involve the sulfated Δ^5 steroids primarily acting via modulating different ion channels and influencing the development of GDM in different directions, mostly diabetogenic progesterone and predominantly anti-diabetic estradiol acting both in genomic and non-genomic way, androgens associated with IR and hyperinsulinemia, neuroactive steroids affecting the pituitary functioning, and cortisol whose production is stimulated by CRH but which suppresses its pro-inflammatory effects. Due to the complex actions of steroids, studies assessing their predominant effect and studies assessing their predictive values for estimating predisposition to GDM are needed.

Key words

Gestational diabetes mellitus • Steroidome • Sex hormone binding globulin • Kisspeptin • CRH

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Introduction

Gestational diabetes mellitus (GDM) is a serious pregnancy complication, in which women without previously diagnosed diabetes develop persistent hyperglycemia during pregnancy. The women with a normal pre-gravid glucose tolerance but manifestation of GDM in pregnancy have subclinical metabolic dysfunction prior to conception compared with women without GDM (GDM-) (Catalano 2014) and the hyperglycemia during pregnancy is generally induced by glucose intolerance due to dysfunction of pancreatic β -cells and in the context with chronic insulin resistance (IR) (Buchanan and Xiang 2005, Xiang *et al.* 2013). Advanced maternal age, obesity and a family history of diabetes are common risk factors of GDM. GDM is regularly associated with macrosomia, birth complications in the infant, increased risk of maternal cardiovascular disease and predisposition to type 2

diabetes mellitus (DM2) in both mother and child (Plows *et al.* 2018). The prevalence of GDM is about 15 %-20 % of pregnancies worldwide with an increasing trend linked to the accelerating obesity (Mirghani Dirar and Doupis 2017, Plows *et al.* 2018, Wang *et al.* 2019). A number of interrelated factors affecting both insulin secretion and resistance are involved in GDM pathophysiology (Vejrazkova *et al.* 2014). Several genetic variants significantly associated with genes related to insulin secretion and insulin receptors were reported in women with GDM (GDM+) (Mirghani Dirar and Doupis 2017). Human pregnancy is also associated with pronounced changes in the levels of a number of bioactive substances including steroids (Vejrazkova *et al.* 2014).

GDM and blood glucose homeostasis

In a fasting state, low glucose levels keep insulin levels low as well, while the levels of the counter-regulatory hormones glucagon, adrenaline and corticosteroids are elevated. Conversely, in a state of satiety, insulin is released from pancreatic β -cells, reducing glucose levels through its uptake in muscle cells and adipocytes as well as by preventing glycogenolysis and gluconeogenesis in the liver (Ropero *et al.* 2008).

While the normal pregnancy is accompanied by lower insulin sensitivity (IS) reduced hepatic insulin extraction, lower glucose effectiveness, higher levels of leptin at lower concentrations of adiponectin, increased lipolysis and higher levels of triglycerides in white adipose tissue (WAT) and by increased WAT expansion, the pregnancy complicated with GDM is additionally associated with increased levels of inflammatory cytokines, higher macrophage infiltration, and pronouncedly increased lipolysis at prominently lower IS. In contrast to healthy pregnancy with mild IR, the pregnancy complicated with GDM exhibit severe IR, hyperglycemia, leptin resistance, hyperlipidemia, release of inflammatory cytokines, and oxidative stress. GDM+ women are unable to compensate for the IR of pregnancy by intensified insulin secretion. However, the mechanisms of β -cell adaptation are not fully understood. (Kautzky-Willer *et al.* 1997, Moyce and Dolinsky 2018).

Early in gestation, insulin secretion increases, stimulating the glucose uptake into adipose tissue to satisfy the rising energy demands in later pregnancy. However, various placental hormones like female sex steroids, cortisol, leptin, placental lactogen, and placental growth hormone induce an IR. This results in slightly elevated blood glucose, which is swiftly transported

across the placenta to fulfill the growing energy needs of the fetus. Furthermore, there is also breakdown of fat stores inducing a further increase in blood glucose as the levels of free fatty acids. The mother recompenses these needs on the account of pancreatic β -cells hypertrophy and augmented GSIS (Plows *et al.* 2018).

In late gestation the maternal system switches to the catabolic phase and IR (associated with stimulated lipolysis, raised free fatty acid (FFA) levels, and hyperlipidemia) is natural but transient. In spite of this transformation, maternal blood glucose remains constant or even slightly declines with advancing gestation primarily due to increased pancreatic secretion of maternal insulin. Even though the peripheral tissues are more insulin resistant in pregnancy at continuing production of hepatic glucose, glucose homeostasis in a healthy pregnancy is reached *via* increasing insulin secretion to overcome the IR (Moyce and Dolinsky 2018). However, in mothers with GDM, β -cells may become damaged due to overproduction of insulin, which permanently exhausts the cells. Furthermore, GDM is also associated with upregulated gluconeogenesis in the liver (Plows *et al.* 2018). While in normal pregnancy the maternal IS returns to pre-pregnancy levels shortly after labor, in GDM+ women, problems frequently persist in the postpartum period and may sooner or later develop to overt DM2 (Buchanan and Xiang 2005, Plows *et al.* 2018, Vejrazkova *et al.* 2014). To maintain euglycemia in the mother, normal pregnancy is accompanied by about 50 % decrease in insulin-mediated glucose disposal and approximately doubled insulin secretion. (Vejrazkova *et al.* 2014).

The β -cell death is reduced in GDM+ women but this phenomenon is linked to compromised insulin production and hyperglycemia and does not contribute to the development GDM. The β -cell dysfunction in GDM+ women is independent of obesity but correlates with the severity of glucose intolerance. While the insulin secretion is insufficient or unaffected in lean GDM+ women (Kautzky-Willer *et al.* 1997), obese GDM+ women exhibit raised increase in insulin response at suppressed IS and hepatic glucose production during insulin infusion with advancing gestation when compared with a matched control group (Catalano *et al.* 1999). The weakening of β -cell function is most apparent in late pregnancy due to growing inability of the endocrine pancreas to satiate the raising metabolic demand (Kenna *et al.* 2016, Saisho *et al.* 2010, Xiang *et al.* 2013).

Without a substantial growth of insulin secretion and independently of baseline age, obesity, diet, physical activity, changes in body weight and additional

pregnancies, GDM+ women exhibit a faster decrease in both β -cell compensation and IS during the 4-year follow-up postpartum when compared with their GDM- counterparts. The more pronounced weakening of the β -cell compensation in GDM+ patients is substantially but not entirely explained (about 50 %) by differences between GDM+ and GDM- groups. Other factors like genetic predisposition to GDM that may contribute to additional explanation of this phenomenon (Kwak *et al.* 2012, Xiang *et al.* 2013). In general, each degree of the glucose intolerance in pregnancy including GDM is a strong predictor for the development of DM2 in future (Kramer *et al.* 2014).

Sex hormone binding globulin

Sex hormone-binding globulin (SHBG) is produced by the liver and its half-life is one week. Insulin is an important regulator of SHBG, inhibiting its production in hepatocytes. Besides the liver, SHBG can also be synthesized by placental trophoblastic cells during pregnancy. SHBG is a glycoprotein that inactivates unconjugated sex steroids and serves as a transporter and reservoir for future use. In addition to insulin, SHBG synthesis is also inhibited by testosterone and prolactin and stimulated by estradiol. (Kopp *et al.* 2001, Zhang *et al.* 2018).

In pregnancy, defective insulin signal transduction receptors and glucose transport proteins were found in the placental tissue of GDM patients. SHBG is involved in the regulation of insulin signaling. Therefore, a decrease in expression may result in a simultaneous decrease in expression of relevant insulin-signaling components in placental tissue and subsequent insulin resistance (Zhang *et al.* 2016).

Up to the third trimester, SHBG levels are reduced in GDM+ patients compared to controls. Low SHBG levels are associated with increased IR and hyperinsulinemia and appear to be the best predictor of GDM. However, the SHBG concentrations are not associated with peripheral IS (see also review (Bartha *et al.* 2000, Vejrazkova *et al.* 2014). Although some authors did not find an association between SHBG and GDM (McElduff *et al.* 2006), most of them demonstrate that low circulating levels of SHBG strongly predict the risk of DM2 (Ding *et al.* 2009, Tawfeek *et al.* 2017) and are also low in GDM+ patients (Faal *et al.* 2019). Moreover, low pre-pregnancy SHBG levels are also tightly associated with a predisposition to GDM (Hedderston *et al.* 2014).

Peptide hormones regulating steroid production

Kisspeptin

Kisspeptins primarily originating in the hippocampal dentate gyrus form a neuropeptide family playing a key role in the initiation of puberty and in female fertility, when kisspeptin ensures pulsatile GnRH release and subsequent secretion of gonadotropins. However, the main source of kisspeptin during pregnancy is the placenta. Kisspeptins, encoded by KISS1 gene (and particularly the main product kisspeptin-54 protein also known as metastin) are potential biomarkers of a number of pregnancy complications and processes including miscarriage, preeclampsia, gestational trophoblastic neoplasia (GTN), fetal development (where kisspeptin provides the brain sex differentiation), and parturition (where kisspeptin stimulates oxytocin secretion). Kisspeptin levels considerably raise during pregnancy (900-fold surge in the first trimester and about 7,000-fold escalation in the third trimester in comparison with non-pregnant women). Interestingly, during pregnancy, kisspeptin mRNA levels do not reflect its serum concentration, with mRNA expression even having a paradoxically descending trend (Hu *et al.* 2019). Kiss1R are expressed in internal, fetal zone of the fetal adrenal (FZFA) from week 8 of gestation until delivery, with kisspeptin levels positively correlated with the growth of fetal adrenal (FA) volume in the second trimester (Katugampola *et al.* 2017).

Kisspeptin and its receptor, Kiss1R, are expressed in the central nervous system, pancreas, adipose tissue, testes, and spleen. A strong expression of Kiss1R mRNA was found in the tissue of the FA and expression of Kiss1R protein was located in FA definitive zone and in the transition zone from week 14 to week 36 of gestation. The increase in Kiss1R expression in mid-gestation between weeks 13 and 22 of gestation is in parallel with the increase in SULT2A1 (converting DHEA to DHEAS) mRNA expression, suggesting that FZFA is the target tissue for kisspeptin, especially in the second trimester. High circulating levels of kisspeptin in the FA suppress Kiss1R expression most probably to ensure optimal regulation of steroid production (Katugampola *et al.* 2017).

ACTH secreted from the fetal pituitary gland is one of the key growth regulators of the FA, however placental CRH and estrogens also play important roles in the FA development. The rapid FA growth and FZFA steroid production during the second trimester are not

accompanied by the ACTH increase. Instead, the CRH levels in human climax near parturition (week 35 of gestation). This indicates that in addition to ACTH, other factors specific to pregnancy also regulate FZFA growth and activity in the second trimester of pregnancy (Katugampola *et al.* 2017).

Kisspeptin stimulates DHEA production in FZFA between week 10 and week 22 of gestation with its effect comparable to that of ACTH or CRH. While the combination of kisspeptin with ACTH has a stimulating effect on DHEAS production in FZFA, its combination with CRH does the opposite. Consequently, it turns out that the resulting FZFA activity is contingent on a combination of factors involving ACTH, kisspeptin, CRH, and estrogens, which are mostly of placental origin (outside ACTH). Like CRH levels, estrogen levels progressively increase with gestational age (Katugampola *et al.* 2017).

Kisspeptin increases (via its receptor GPR54) GSIS in β -cells of pregnant women. Therefore, low kisspeptin levels are associated with the risk of GDM. Kisspeptin thus provides the placental signal needed to adapt β cells during pregnancy for maintaining glucose homeostasis (Bowe *et al.* 2019, Simpson *et al.* 2018). The relationship between kisspeptin and insulin secretion is independent of other key determinants of glucose homeostasis, such as IS, glucose stress test results, adiposity, age and gender (Andreozzi *et al.* 2017).

CRH

Outside of pregnancy, corticoliberin (CRH) is the hypothalamic hormone controlling, through stimulation of ACTH secretion in the pituitary gland, cortisol production in the adult adrenal gland. The hypothalamic–pituitary–adrenal (HPA) axis in non-pregnant subjects is based on a negative feedback loop between the final active hormone cortisol, ACTH and CRH. In pregnancy, after the luteo-placental shift, CRH is primarily expressed in the human placenta, creating a positive feedback loop between cortisol and CRH, while ACTH production stagnates. CRH directly stimulates the production of Δ^5 steroid sulfates in the fetal zone of the fetal adrenal (FZFA) and cortisol synthesis in the fetal adrenal transition zone. As for CRH's key physiological role in pregnancy, some authors hypothesize the so-called “placental clock” (Hill *et al.* 2010a). This most sophisticated hypothesis to date on the role of placental CRH in pregnancy in all probability explains how this proteohormone determines the duration of pregnancy and the start of labor (McLean *et al.* 1995,

Sandman *et al.* 2006, Smith and Nicholson 2007). A number of experimental data support this concept, yet some important questions remain unanswered (Gangestad *et al.* 2012). Almost all placental CRH (>99 %) passes into the maternal circulation and only less than 1 % of placental CRH passes into the fetus (Campbell *et al.* 1987). CRH levels in the maternal circulation in the 3rd trimester of pregnancy exceed CRH levels in early pregnancy by approximately 3 orders of magnitude and are more than an order of magnitude higher than CRH levels in the fetus (Gangestad *et al.* 2012, Goland *et al.* 1994, Goland *et al.* 1988, Sasaki *et al.* 1987). Therefore, CRH levels in the maternal circulation are almost exclusively of placental origin (Gangestad *et al.* 2012, Smith *et al.* 2001).

Some authors have raised the question of why the placenta secretes the vast majority of CRH into the maternal bloodstream and proposed a different concept explaining CRH's function (Gangestad *et al.* 2012). However, these authors did not completely reject the concept based on the “placental clock”. Unlike previous studies that considered cortisol to be harmful to the fetus, they suggest that cortisol, on the other hand, is beneficial to the fetus because it stimulates glucose levels via liver gluconeogenesis and maintains insulin insensitivity leading to a reduction in peripheral glucose consumption, thereby providing a greater flow of glucose through the placenta to the fetus (Gangestad *et al.* 2012). Gangestad *et al.* suggested that cortisol works in the same way as placental hormones (human placental lactogen (hPL) and human growth hormone to ensure glucose uptake in the placenta and subsequent glucose transport to the fetus (Gangestad *et al.* 2012). Furthermore, Smith *et al.* (Smith *et al.* 2001) reported that peripheral administration of CRH increases fat oxidation. While human growth hormone and hPL have direct effects, placental CRH acts by stimulating maternal cortisol production and placental CRH production increases in response to increasing fetal energy needs. For example, starvation increases CRH production and shortens pregnancy duration (Herrmann *et al.* 2001). For this reason, CRH production is also higher in multiple pregnancies to meet greater demands for maternal energy supply (Gangestad *et al.* 2012). Placental CRH also mediates insulin secretion (Schmid *et al.* 2011) and proliferation of pancreatic β -cells (Mack and Tomich 2017). On the other hand, overly high CRH levels in pregnant women may induce a pathological grade IR and hyperglycemia leading to GDM (Wolfe *et al.* 1988).

Steroids, steroidogenic enzymes, and steroid-related receptors

Local islet steroidogenesis and pancreatic steroid receptors

There are a number of results documenting the ability of mammalian pancreas to synthesize steroids (Mauvais-Jarvis 2016). This organ expresses steroidogenic enzymes such as cholesterol desmolase (CYP11A1), responsible for the first, limiting step in steroidogenesis, type 3 17 β -hydroxysteroid dehydrogenase (HSD17B3) converting inactive androstenedione to active testosterone, type 1 17 β -hydroxysteroid dehydrogenase (HSD17B1) transforming inactive estrone to active estradiol, and type 12 17 β -hydroxysteroid dehydrogenase (HSD17B12), which is specifically pancreatic and works in the opposite direction to HSD17B1. The steroid C17-hydroxylase, C17,20 lyase (CYP17A1) is active in the β cells in both hydroxylase and lyase steps and is co-localized with CYP11A1. Furthermore, the CYP11A1 is also co-localized with 3 β -hydroxysteroid dehydrogenase- $\Delta^{4/5}$ isomerase HSD3B and the β -cells also express aromatase (CYP19A1) and 5 α -reductase (SRD5A). Therefore, the pancreatic tissue is capable to synthesize several active steroid hormones such as progesterone, testosterone, 5 α -dihydrotestosterone, estradiol, and neuroactive steroids, whose concentrations in the pancreatic tissue roughly exceed their blood concentrations by an order of magnitude. The steroids above may then act on estrogen and androgen receptors in intracrine and paracrine ways and may be inactivated in the same way. (Mauvais-Jarvis 2016, Morales *et al.* 1999, Robles-Diaz and Duarte-Rojo 2001). Progesterone receptors are expressed in the pancreas, predominantly in cells that produce glucagon. In addition, pancreatic tissue also expresses androgen receptors (Robles-Diaz and Duarte-Rojo 2001).

Of the steroids synthesized in the pancreas, estradiol, progesterone, and androgens are involved in stimulating insulin secretion. At physiological concentrations, estradiol increases insulin secretion in the presence of glucose by acting on the membrane receptor that closes the ion K_{ATP} channels. In addition, treatment with estradiol successfully prevents the development of DM2. In mice, treatment with progesterone leads to higher insulin production. In rats, the 5 α -reduced metabolites of C19 steroids (epiandrosterone and 5 α -dihydrotestosterone) reduce the harmful effects of interleukin 1b and NO-mediated damage on insulin secretion (Robles-Diaz and Duarte-Rojo 2001).

Δ^5 Steroids

The main source of Δ^5 steroids outside pregnancy is primarily the adrenal cortex. Whereas C21 Δ^5 steroids (pregnenolone, 17-hydroxypregnenolone) are synthesized in *zona fasciculata* similarly to cortisol, the source of C19 Δ^5 steroids (DHEA, androstendiol) is primarily *zona reticularis*. In men, a smaller proportion of C19 Δ^5 steroids may also arise as intermediates for testosterone synthesis. In both sexes, however, both C21 and C19 Δ^5 steroids are produced in the human adrenal gland primarily in the form of relatively hydrophilic and well soluble sulfates and consequently also circulate in blood in concentrations 1-3 orders of magnitude higher than their free analogues (Hill *et al.* 1999, Mitamura *et al.* 2003, Sulcova *et al.* 1997, Tagawa *et al.* 2004). The ratio of C19 to C21 Δ^5 adrenal steroids grow with growing cytochrome B5 (CYB5) activity in adrenal cortex (Rege *et al.* 2016).

In contrast to non-pregnant subjects, the main source of pregnancy steroids is the fetal adrenal cortex primarily producing the sulfates of Δ^5 steroids (pregnenolone, 17-hydroxypregnenolone, DHEA, androstenediol). These steroids circulate in the fetal blood in concentrations mostly exceeding 1-3 orders of magnitude their values outside pregnancy. Only the levels of DHEA sulfate are lower in pregnancy than outside pregnancy. The sulfates of the Δ^5 steroids are then easily transported to the placenta, even at excessive concentrations, where they are first swiftly desulfated by steroid sulfatase (STS) and then readily converted by type 1 3 β -hydroxysteroid dehydrogenase (HSD3B1) to the corresponding Δ^4 steroids (progesterone, 17-hydroxyprogesterone, androstenedione, testosterone) as activities of the aforementioned enzymes in placenta are extremely high. Alternatively, since the placenta has only very low activity of CYP17A1, there is no significant placental conversion of 17-hydroxy-C21 steroids to their C19 analogues as well as conversion of 17-deoxy-C21 steroids to their 17-hydroxy- analogues. The Δ^4 steroids (mainly androstenedione) resulting from the released C19 Δ^5 steroids (DHEA, androstenediol) are then suitable substrates for the biosynthesis of estradiol, as the placental activity of CYP19A1 that catalyzes this step is also very high (2015, Hill *et al.* 2010a).

While the origin of fetal Δ^5 steroids is detailed above, in the maternal compartment, the major sources of C19 Δ^5 steroids androgens are *zona reticularis* in the maternal adrenal cortex and also the maternal ovaries. A baboon study showed an inhibitory effect of estradiol on the production of DHEA/DHEAS in *zona reticularis* in pregnant women, consistent with the observed

decrease in DHEAS production in maternal blood. Even with placenta maintenance, fetal removal (fetectomy) caused a decrease in estradiol levels in the maternal circulation, while increasing levels of DHEA/DHEAS. In addition, estradiol substitution by addition to the maternal circulation inhibits the decrease in maternal DHEA/DHEAS levels at unchanged DHEA/DHEAS metabolic clearance rates. This confirms that the sources of maternal estradiol are sulfated C19 steroids synthesized in the FZFA and transformed by placenta into estradiol, which then enters the maternal circulation (Hill *et al.* 2010a, Makieva *et al.* 2014). At the same time, it shows that the decrease in C19 steroids in the maternal *zona reticularis* depends primarily on the production of C19 steroids in FZFA (Makieva *et al.* 2014).

Although the Δ^5 do not operate in a genomic way they are effective as modulators of ionotropic receptors (especially sulfates) and affect numerous physiological functions particularly in human immune system (Sterzl *et al.* 2003, Sterzl *et al.* 1999, Tagawa *et al.* 2004, Tagawa *et al.* 2002, Tagawa *et al.* 2001, Tagawa *et al.* 2000).

Steroid levels in pregnant women and fetuses often range in excess of 1-3 orders of magnitude of these concentrations outside pregnancy (Hill *et al.* 2011, Hill *et al.* 2014). Simultaneously, steroids affect both IS in tissues and insulin secretion in pancreatic β -cells (Vejrazkova *et al.* 2014), especially at excessive concentrations that are common during pregnancy (Hill *et al.* 2011, Hill *et al.* 2014). For example, pregnenolone sulfate (PregS) stimulates insulin secretion by positively modulating non-selective type 3 melastatin ion channels (TRPM3), where this steroid conjugate opens these ion channels for Ca^{2+} penetration even at concentrations common outside pregnancy (Naylor *et al.* 2010). At the same time, PregS levels during pregnancy in the mother and fetus are in excess of those outside pregnancy by 1 and 2 orders of magnitude, respectively (Hill *et al.* 2011, Hill *et al.* 2014). Dehydroepiandrosterone sulfate (DHEAS) also has a similar effect on TRPM3 like PregS (Majeed *et al.* 2010) but DHEAS levels in pregnancy are either comparable or rather lower than outside pregnancy. Activation of TRPM3 channels by the aforementioned steroids leads to the activation of rapidly accelerated fibrosarcoma (RAF) kinases and extracellular signal-regulated kinases (ERK) and subsequently insulin secretion. PregS also activates signal cascades involving the amplified expression of activator protein 1 (AP-1) and transcription factors Egr-1, c-Jun, and c-Fos in the pancreatic β -cells. Voltage gated L-type Ca^{2+} channels are also involved in β -cell activation, but their expression

is insufficient to activate the transcription factors mentioned above (Lesch *et al.* 2014). In addition to TRPM3 modulation, PregS may also be involved in insulin secretion through positive modulation type 1 melastatin ion channels (TRPM1) (Lambert *et al.* 2011). Furthermore, PregS and another also abundant steroid conjugate isopregnanolone sulfate (progesterone metabolite) (reaching micromolar concentrations during pregnancy) inhibit specific proton-activated outwardly rectifying anion channels (PAORAC). This may further contribute to increased β -cell activity (Drews *et al.* 2014).

Moreover, PregS (at concentrations common in fetal circulation) inhibits nicotine-acetylcholine receptors (nAChR) and inhibits the influx of Ca^{2+} and Na^+ ions into β -cells, thereby suppressing insulin secretion (Kudo *et al.* 2002). DHEAS, independent of pregnancy, has a similar effect (Paradiso *et al.* 2000). The nAChR containing α_7 and β_2 subunits are thus involved in maintaining energy homeostasis and IS (Somm *et al.* 2014). The data above indicates that in addition to stimulating insulin secretion, the sulfated Δ^5 steroids may also reduce insulin secretion and peripheral IS but a question remains which direction prevails regarding the resulting influence on GDM.

Like the effects of PregS, the effects of C19 Δ^5 steroids are not clear-cut in relation to the pathophysiology of DM2 (and GDM). On one hand, already a single dose of DHEA induces alterations in insulin receptor, IRS-1, IRS-2, and Act-1 protein levels while increasing the weight of pancreatic β -cell mass and GSIS (Medina *et al.* 2006, Morimoto *et al.* 2011). Another study reports increased GSIS in DHEAS-treated rat insulinoma RINm5F-cells (Liu *et al.* 2006a). Like some of its reduced metabolites, DHEA mitigates the cytotoxic effect of interleukin 1b and its harmful effect on glucose transport (Liu *et al.* 2006a). DHEA supplementation in female rats after ovariectomy also improves GSIS disrupted by a high-fat diet (Veras *et al.* 2014). Several animal studies during feeding indicate that DHEA/DHEAS reduce glycaemia, suggesting that intestinal factors are likely to contribute to these results (Liu *et al.* 2006a).

On the other hand, three-day cultivation with BRIN-BD11 rat pancreatic cells with either DHEAS (30 μM) or DEX (100 nM) diminishes the total number of cells, reduces cell viability, reduces insulin content, and impairs insulin secretion and responsiveness in these cells. The DHEAS application reduces insulin secretion in BRIN-BD11 cells by roughly one half, with slightly less pronounced reductions in high glucose levels (16.7 mM) compared to low (1.1 mM) or mean glucose levels (5.6 mM). Insulin response remains unchanged

after DHEAS administration at low glucose concentrations, decreases by about a fifth at moderate concentrations, and remains almost unchanged again at high glucose concentrations. Insulin secretion decreases substantially after DHEAS administration both in the presence of various secretagogues (KCl 30 nM, alanine 20 mM, Ca^{2+} 7.68 nM forskolin 25 μM , PMA 10 nM) as well as without them (Liu *et al.* 2006b). In rat INS-1 cells, DHEA (through a rapid non-genomic mechanism) weakens Ca^{2+} release and inhibits insulin secretion stimulated by the insulin agonist carbachol. These results support the existence of a specific plasma membrane receptor for DHEA that mediates this signal transduction pathway by G-proteins (Morimoto *et al.* 2011).

Progesterone

The origin of progesterone depends largely on gender and, in women, on menstrual cycle and pregnancy. While low levels of circulating progesterone are more likely to be of adrenal origin in males and females in the follicular phase of the cycle, women in the luteal phase are clearly dominated by progesterone of ovarian origin, and after the luteo-placental shift of pregnancy, progesterone is almost exclusively of placental origin in both mother and fetus (Kancheva *et al.* 2007). Two routes are involved in the synthesis of pregnancy progesterone, although biosynthesis of pregnancy progesterone takes place in placenta in both cases. The first route depends on the transport of maternal LDL cholesterol into the placenta and its subsequent conversion to pregnenolone, which is catalyzed by the enzyme cholesterol desmolase (CYP11A1) and then on the conversion of pregnenolone to progesterone catalyzed by HSD3B1. The second route of progesterone formation depends on the delivery of significant amounts of pregnenolone sulfate from the adrenal gland of the fetus (circulating in micromolar concentrations in the fetus), its subsequent hydrolysis catalyzed by placental STS, and further conversion of the released pregnenolone, which is identical to the first route of progesterone formation. Because during transplacental steroid passage towards the fetus, oxidative conversions towards oxo-groups in positions C3, C17 and C20, are generally preferred, levels of progesterone (20-oxo steroid) in fetal blood are at micromolar concentrations, whereas in maternal blood progesterone circulates only in concentrations of hundreds of nanomoles. Conversely, the proportion of 20 α -dihydroprogesterone (20 α -hydroxy steroid) is higher in the mother compared to the fetus (Hill *et al.* 2010a, Hill *et al.* 2010b, Hill *et al.* 2011).

The primary role of progesterone lays in female

reproductive functioning during the menstrual cycle and in pregnancy. Progesterone is important as a pregnancy stabilizing steroid and also significantly affects glucose homeostasis in pregnancy. Progesterone (whose receptors are present in β -cells) is primarily a diabetogenic steroid that acts by suppressing the expression of type 4 glucose transporter (GLUT-4). Increased endocrine pancreatic activity in the 3rd trimester of pregnancy is related to increased progesterone levels and is an adaptive response to the increasing energy needs of a fetus requiring increased glucose and lipid intake. Progesterone works in combination with other hormones such as hPL, estradiol or cortisol (Bernard-Kargar and Ktorza 2001, Morimoto *et al.* 2011, Wilcox 2005). Progesterone is likely to be the central regulator of β -cell proliferation in response to metabolic challenges such as IR (Branisteanu and Mathieu 2003, Mauvais-Jarvis 2016). For example, the therapeutic use of its metabolite 17-hydroxyprogesterone increases the risk of GDM (Rebarber *et al.* 2007). Furthermore, progesterone antagonists suppress glucose levels. Pancreatic islets from progesterone KO mice were reported to be larger and produce more insulin due to β -cell proliferation and β -cell weight gain. (Picard *et al.* 2002).

Progesterone, unlike sulfated Δ^5 steroids, inhibits the permeability of TRPM3 channels already at levels common in the luteal phase of the menstrual cycle (Majeed *et al.* 2012). At the same time, the progesterone levels in maternal and fetal circulation are still 1 to 2 orders of magnitude higher (Hill *et al.* 2011, Hill *et al.* 2014). Animal experiments indicate that nuclear progesterone receptors may also be involved in GDM pathophysiology (Picard *et al.* 2002). Unlike inhibition of action in TRPM3 channels, progesterone (at concentrations common in the luteal phase) and also DHEA (at physiological concentrations) activate purinergic ionotropic P2X receptors (De Roo *et al.* 2010, De Roo *et al.* 2003). At the same time, subtypes of P2X receptors are expressed in different tissues, including β -cells, and their activation facilitates (at low glucose concentrations) the influx of Ca^{2+} and Na^+ ions, with subsequent depolarization of the cell membrane and secretion of insulin (Burnstock 2014).

Administration of progesterone and estradiol to female rats results in an increase in GSIS while increasing glucose tolerance (Mauvais-Jarvis 2016, Nielsen 1984). In MIN6 cells (mouse insulinoma cells) and RIN 1046-38 cells (rat insulinoma cells), progesterone increases glucokinase activity (GK). Thus, an increase in both basal and glucose-stimulated insulin secretion during pregnancy may be related to high levels

of progesterone. However, these effects may limit the ability of lactogenic hormones to increase GSIS in late gestation (Morimoto *et al.* 2011).

However, the effects of progestogens on GSIS are contradictory as progesterone rapidly and specifically inhibits GSIS ($IC_{50}=10\ \mu\text{M}$ at 8.4 mM glucose) in rat β -cells. On the outer surface of the β -cell plasma membrane, progesterone reduces the inlet of Ca^{2+} ions into the cell through the blockade of L-type voltage-gated Ca^{2+} channels (but does not inhibit insulin secretion stimulation in the absence of Ca^{2+}) (Straub *et al.* 2001). Several authors report that unlike prolactin and placental lactogen, which stimulate insulin secretion, progesterone suppresses their effects by down-regulation of β -cell function in the later stages of gestation (Kawai and Kishi 1999, Morimoto *et al.* 2011, Shao *et al.* 2004, Weinhaus *et al.* 2000).

Testosterone

Besides massive testosterone synthesis in adult male testicles, this active androgen can also be synthesized in both sexes in the adrenal *zona reticularis*, which in addition to weak or inactive androgens such as DHEA, DHEA sulfate, androstenediol, androstenediol sulfate, and androstenedione can also synthesize testosterone, because the enzyme that converts androstenedione into testosterone (AKR1C3) is also active in this adrenal zone (Nakamura *et al.* 2009). In addition, a substantial portion of testosterone in both sexes may arise from adrenal steroids (mainly DHEA) that are converted to testosterone in peripheral tissues. Another source of testosterone in pregnancy may be the testicles of the male fetus, but these are only active in the first trimester of pregnancy in terms of testosterone production. Although testosterone is a key male testicular hormone, it also affects many physiological functions in women, both in pregnancy and beyond (Labrie 2010, Labrie 2015, Labrie *et al.* 2003) and testosterone production in the fetal testes in the first trimester of pregnancy provides the hormonal control of fetal sexual development (Huhtaniemi 1994).

Overproduction of testosterone in women may indicate a number of pathologies, mainly related to adrenal and/or ovarian disorders. Hyperandrogenism is generally associated with predisposition to β -cell dysfunction in women (Diamond *et al.* 1998, Navarro *et al.* 2015). Testosterone increases GSIS in β -cells by binding to extranuclear androgen receptors that induce cAMP production and activate cAMP-dependent protein kinase A in a similar way to GLP-1 (Mauvais-Jarvis 2016)). In animal models, 5 α -DHT infusion in females

leads to hyperinsulinemia and IR dependent on the AR via a cAMP- and mTOR- dependent pathway. In β -cells, 5 α -DHT induces increased mitochondrial respiration associated with increased oxygen consumption and subsequent increased oxidative damage to these cells (Navarro *et al.* 2018). A positive correlation was found between maternal and fetal testosterone levels in male neonates. Testosterone levels were higher in newborns in mothers with GDM+ (Morisset *et al.* 2013). Even in non-diabetic populations of pregnant women, both total and free testosterone levels are positively correlated with fasting C-peptide levels (Ackerman *et al.* 2013) and abnormal increases in androgen levels during pregnancy are associated with a high risk of developing GDM (Makieva *et al.* 2014). In a study involving patients with GDM+ and appropriate controls, a significant positive relationship of circulating testosterone to insulin response following oral glucose administration was observed, while a negative correlation between testosterone and IS was observed.

Estradiol

In postmenopausal women, all sex steroids, including estradiol, are produced intracellularly in peripheral tissues (Labrie 2015), while in men, estradiol is produced predominantly by peripheral conversion of testicular and adrenal androgens (testosterone and androstenedione) in adipose tissue that exhibits relatively high CYP19A1 activity (Belanger *et al.* 2002, Labrie 2010, Rubinow 2018). In the luteal phase, most estrogens are produced in the ovaries, and in pregnancy, estradiol is almost exclusively a product of the placenta as mentioned above (Hill *et al.* 2010a, Simpson 2003).

Estrogens in postmenopausal women and men are not drivers of estrogen action, they are more reactive than proactive, since in these cases the circulating estrogen originates from non-gonadal sites, acts locally and only then enters circulation. Therefore, the circulating estrogens levels here rather reflect their effects, but are not their controlling element (Berkane *et al.* 2017, Simpson 2003).

A completely different situation occurs in women in the luteal phase and even more so in pregnancy, where the sex hormone estradiol affects and/or provides a range of physiological functions and is involved in the pathophysiology of various disorders (Berkane *et al.* 2017, Hill *et al.* 2010a, Simpson 2003).

During pregnancy, insulin levels in plasma increase in tandem with estradiol increase (Roperio *et al.* 2008). Estrogens participate in both rapid non-genomic and slow, genomic regulation of insulin secretion. As for the non-genomic effects of estrogens, estradiol binds to

estrogen receptors (ER) such as type α ER (ER α) and type β ER (ER β), modulating the transcription of target pancreatic genes (Morimoto *et al.* 2011). While ER α are involved in the production of insulin and maintenance of nutritional homeostasis, ER β is involved in increasing the GSIS. G protein-coupled ER (GPER), which is located in an extranuclear position, is involved in the regulation of GSIS and in the survival of β -cells (Mauvais-Jarvis 2016). ER α , which is expressed in pancreatic islets instigates activation of MAPKs (mitogen-activated protein kinases) (Sutter-Dub 2002) and also induces expression of glucose transporter 4 (GLUT4). The GLUT4 plays a key role in glucose homeostasis and is a limiting step in the uptake of glucose in skeletal muscles that is induced by insulin.

In addition to the slow genomic effect, estradiol also produces rapid non-genomic effects through activation of the membrane-initiated signal pathways. The endocrine pancreatic cell response includes estradiol binding to membrane receptors followed by a change in membrane potential, an increase in Ca²⁺ influx into the cell, and the activation of protein kinase, cyclic guanosine monophosphate (cGMP) dependent kinase, and mitogen-activated protein kinase (Mauvais-Jarvis 2016, Morimoto *et al.* 2011, Sutter-Dub 2002). In physiological concentrations, estradiol increases cGMP levels in β -cells as well as the activity of the protein kinase G (PKG), which subsequently reduces K_{ATP} activity through the rapid and reversible closure K_{ATP} channels. In synergy with increased glucose levels, it also depolarizes the cell membrane, triggering electrical activity and influx Ca²⁺ into the cell via activation of voltage-gated Ca²⁺ channels, leading to Ca²⁺ oscillations and consequently to pulsatile insulin secretion and the activation of Ca²⁺-dependent transcription. In glucagon synthesizing α -cells, estradiol abolishes Ca²⁺ oscillations generated by low glucose levels, reducing glucagon secretion (Nadal *et al.* 1998, Ropero *et al.* 2002) (see also reviews (Morimoto *et al.* 2011, Ripoll *et al.* 2008, Ropero *et al.* 2008)). Moreover, estradiol inhibits the permeability of voltage dependent potassium channels (KQT-like subfamily member1, KCNQ1), which also activate β -cells (Moller and Netzer 2006). At the same time, estradiol levels in the maternal circulation exceed by approximately two orders of magnitude their value in the luteal phase of the menstrual cycle (Hill *et al.* 2011). The L-type Ca²⁺ channels may also participate in the regulation of peripheral IS (Xu *et al.* 2004). In addition to insulin secretion, estrogens stimulate energy consumption and food intake by affecting the CNS, increasing glucose uptake in muscles and adipocytes, and decreasing liver gluconeogenesis.

(Mauvais-Jarvis 2011).

Concerning the associations between estrogens and GDM development, reduction of GLUT4 expression contributes to the reduction of IS (Morimoto *et al.* 2011). In addition, ER α are involved in modulation of hepatic IS because ER α KO mice show severe hepatic IR and undesirable changes in skeletal glucose uptake. Absence of ERs is associated with IR and glucose intolerance and leads to adipocyte hyperplasia and hypertrophy in WAT but not in brown adipose tissue (Ropero *et al.* 2008). Both ER KO and aromatase KO mice are obese and insulin resistant see review (Ropero *et al.* 2008). Long-term exposure to estradiol physiological levels, on the other hand, results in increased expression of the insulin gene and increased release of insulin with Era (Alonso-Magdalena *et al.* 2008). ER α also operate at the central level as their disruption in the ventromedial nucleus of the hypothalamus of female mice leads to increased visceral adiposity, hyperphagia, hyperglycemia, and impaired energy exposure (Ropero *et al.* 2008). The lack of estradiol in female rats results in reduced expression of the gene for K_{ATP} resulting in a decreased insulin secretion, which may be restored by estradiol replacement (Choi *et al.* 2005, Morimoto *et al.* 2001).

Neuroactive steroids

Neuroactive steroids affecting ion channel permeability include some of the steroids discussed above, such as sulfates of pregnenolone and DHEA, or estradiol. However, neuroactive steroids also include a group of 5 α / β -reduced progesterone metabolites, which are formed by conversion of progesterone mainly in the placenta and liver of both the fetus and the mother. High levels of these metabolites reflect extreme levels of progesterone in pregnancy, achieving pharmacologically effective concentrations at a number of receptors, in the direction of neuro-inhibition as well as neuro-excitation, depending on the 3 α - or 3 β -position of the hydroxy-group and the sulfated or non-sulfated form of the steroid (Hill *et al.* 2010a, Hill *et al.* 2011, Hill *et al.* 2014).

The 5 α / β reduced progesterone metabolites could also be involved in the regulation of glucose homeostasis in pregnancy by the following mechanisms. It is known that estradiol produces a rapid opposite effect on the Ca²⁺ influx into the pancreatic β - and α -cells. Unlike β -cells, estradiol inhibits the Ca²⁺ influx oscillations and subsequently glucagon secretion in pancreatic α -cells (Nadal *et al.* 2004). However, the β -cells produce significant amounts of aminobutyric acid (GABA), resulting in GABA receptor type A activation

(GABA_AR) and subsequent inhibition of glucagon secretion in pancreatic α -cells (Jin *et al.* 2013). Meanwhile, insulin negatively modulates GABA_AR via an ERK-dependent negative feedback loop mechanism (Bansal *et al.* 2011). The GABA increases insulin secretion at low concentrations but inhibits it at higher concentrations (Dong *et al.* 2006). GABAergic progesterone metabolites such as allopregnanolone and pregnanolone (at concentrations common in the circulation of the fetus and mother) increase the permeability of GABA_AR receptors for Cl⁻ ions (Fodor *et al.* 2005, Hill *et al.* 2010a, Hill *et al.* 2010c, Hill *et al.* 2011, Hill *et al.* 2014, Park-Chung *et al.* 1999). Conversely, sulfated Δ^5 steroids (at physiological concentrations) are their antagonists (Gartside *et al.* 2010, Mchedlishvili and Kapur 2003, Park-Chung *et al.* 1999).

It is therefore likely that these progesterone metabolites could also influence glucose homeostasis in pregnancy and therefore the development of GDM. The same could be true in another case. It has been described that low glucose levels induce increased permeability of AMPA/kainate receptors for Ca²⁺ ions and opening of voltage dependent Ca²⁺ channels, stimulating glucagon secretion (Cabrera *et al.* 2008). While PregS and allopregnanolone inhibit the AMPA/kainate receptors (Sun *et al.* 2005, Yaghoubi *et al.* 1998) and hence glucagon secretion, estradiol acts as their antagonist (Gu and Moss 1996). In this context, it should be noted that levels of both pregnenolone sulfate and allopregnanolone are substantially elevated during pregnancy (Hill *et al.* 2010a, Hill *et al.* 2010c, Hill *et al.* 2011, Hill *et al.* 2014). To conclude, the influence of neuroactive steroids on the secretion of endocrine pancreatic hormones is undoubtedly worth considering in the context of GDM pathophysiology.

GDM, cortisol, and inflammation

Cortisol is commonly known as the most important human glucocorticoid produced in the *zona fasciculata* of the adult adrenal cortex and its production is controlled by the hypothalamic-pituitary-adrenal (HPA) axis. Cortisol is also well known to suppress the immune response and affect glucose homeostasis. The fetus produces cortisol in an analogous zone known as *transition zone* of the fetal adrenal, but its production largely depends on placental CRH, with which cortisol can generate a positive feedback loop as was describe above (Hill *et al.* 2014).

Besides pregnancy related tissues such as fetal adrenal and placenta, the expression of mRNA for CRH

and CRH-receptor type 1 (CRHR1) and of protein for CRHR1 was found in rat and human pancreatic islets and rat insulinoma cells. Furthermore, mRNA for HSD11B1 and HSD11B2 and protein for HSD11B1 were reported in in rat and human pancreatic islets and insulinoma cells (Schmid *et al.* 2011). Stimulation of CRHR1 and GHRH receptors suppresses HSD11B1 while simultaneously upregulating HSD11B2 in insulinoma cells. The effect is therefore a shift the balance away from active glucocorticoid towards its inactive form (Schmid *et al.* 2011).

Pregnancy is associated with persistent mild pro-inflammatory state and elevated glucose levels. Excessive CRH levels in the mother's blood stimulate the placental production of pro-inflammatory cytokines such as TNF α , IL-1 β , IL-6, and IL-8. While CRH binding to CRH receptors type 1 (CRH1) produces a pro-inflammatory effect, the CRH binding to CRH receptors type 2 (CRH2) does the opposite. At the same time, however, cortisol, whose levels are stimulated by CRH, has anti-inflammatory effects that are associated with an increase in IR beyond the cytokine signaling pathways. This means that cortisol can simultaneously mitigate the pro-inflammatory effects of CRH (Gangestad *et al.* 2012). In terms of the role of cortisol in pathophysiology, it is important that increases in stress hormone levels and stress adaptation disorders may be associated with the pathogenesis of GDM (Feng *et al.* 2020).

Concluding remarks and future prospects

As GDM is both a frequent and serious pregnancy complication, steroid levels in pregnancy are extremely elevated and their role in pregnancy is crucial, this review focuses on the role of steroids and some steroid associated substances in the pathophysiology of GDM. Of these substances, the SHBG in particular plays an important role. Low levels of this binding protein synthesized in the maternal liver are associated with IR and hyperinsulinemia, while also well predicting a predisposition to GDM.

Other important agents involved in the pathophysiology of GDM are placental peptide hormones such as kisspeptin and CRH, which play an important role beyond pregnancy, but are synthesized here in considerably smaller amounts in the hypothalamus. These hormones have their receptors in tissues that affect both the course and termination of pregnancy, such as the fetal adrenal gland, as well as in tissues associated with GDM pathophysiology, such as pancreas. These substances therefore substantially influence the synthesis of pregnancy steroids and may also be involved in the

pathophysiology of GDM.

Steroids themselves, whose biosynthesis in pregnancy is mainly provided by the fetal adrenal glands, placenta, maternal adrenal glands, and both maternal and fetal livers, are also synthesized in limited amounts directly in the pancreas and may influence the development of GDM. These substances involve sulfates of Δ^5 steroids primarily acting by modulating different ion channels and influencing the development of GDM in different directions, predominantly diabetogenic progesterone but predominantly anti-diabetic estradiol, acting both in genomic and non-genomic way, androgens associated (in a similar way to progesterone) with IR and hyperinsulinemia, neuroactive steroids affecting the pituitary functioning and cortisol, the production of which is stimulated by CRH but which also suppresses its pro-inflammatory effects.

However, due to the complex effect of a number

of steroids, steroidomic studies assessing their predominant or resulting effect are desirable in the future, similarly to studies assessing their predictive values for estimating predisposition to GDM.

Conflict of Interest

There is no conflict of interest.

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