Perinatal reproductive endocrinology in singletons and twins

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General introduction and outline of the thesis

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General introduction

Over the last decades there has been a shift in thinking about the role of nurture versus nature and the way this influences a growing human being. Since Barker propagated the notion that 'The womb may be more important than the home', the intra-uterine environment has been considered more and more important as a possible starting point for development of diseases later in life [1]. As demonstrated in the Dutch famine cohort studies, the fetus adapts to a limited supply of nutrients and in doing so it permanently alters its physiology and metabolism. Although these adaptations enable the fetus to continue to grow they may nevertheless have adverse consequences for health in later life [2]. Under-nutrition during gestation, depending on the trimester of exposure, may result in a higher prevalence of coronary heart disease, type 2 diabetes, bronchitis, altered lipid profile, depression and schizophrenia in the offspring [3]. This underlines the importance of a stable intra-uterine environment which is needed for a fetus to develop. Remarkably, women exposed to famine have an increased number of children and more often have multiple pregnancies compared to women born before or conceived after famine [4]. Hormones larger than 7-12 kDa are not able to pass the placenta [5]. Steroid and thyroid hormones do cross the placenta but are metabolized en route. Endocrine fetal development is therefore almost completely under autonomous control. Maternal glucocorticoids normally do not reach the fetus, because of the placental enzyme 11β-hydroxysteroid dehydrogenase, which converts active glucocorticoids into inactive products. However, studies in adult men and women born small for gestational age, demonstrated high fasting cortisol concentrations. The underlying mechanisms described are activation of the hypothalamic-pituitary-adrenal axis (stimulation by exogenous adrenocorticotrophin hormone) and increased cortisol responses to psychosocial stress. Deficiencies in the barrier enzyme, potentially increasing fetal glucocorticoid exposure, can arise in association with maternal stress, malnutrition and disease [6].

With regard to the male reproductive axis, gonadal differentiation begins at 7 weeks of gestation in the presence of the SRY gene. Testicular development starts with differentiation of Sertoli cells, which enlarge and make contact with each other in order to form the seminiferous tubes. Germ cells, which are spermatogenic precursors, are completely enveloped by the growing seminiferous cords. Due to tight junctions between Sertoli cells the blood-testis barrier is formed which is necessary to prevent an auto-immune reaction to spermatogonia [7]. At the end of the 8th week Leydig cells are visible and able to produce testosterone, which is necessary for maintaining the Wolffian system and masculinisation of the fetus. The Wolffian ducts transform into the excretory system which eventually is capable of ejaculation (epididymis, vas deferens, seminal vesicles) [7]. Dihydrotestosterone is necessary for the external male urogenital tract (prostate, scrotum, urethra and the penis) to develop adequately [5]. Aromatase activity is low in the fetal testis, suggesting that estrogens do not contribute to fetal testicular testosterone concentrations [8]. As early as 10 weeks of gestation gonadotropins are detectable in the fetal pituitary and a few weeks later in serum as well [9]. The SRY gene activates anti-Mullerian hormone gene expression, resulting in regression of the Mullerian duct system. AMH is produced in Sertoli cells and reaches the Mullerian ducts largely by diffusion [10]. Between 10 and 12 weeks of gestation the number of immature Leydig cells and testosterone production increases reaching their peak around mid-gestation and decrease again thereafter. Around 2-3 months after birth testosterone concentrations rise again, together with inhibin B, LH and FSH which is often referred to as the 'mini-puberty', which seems to play a role in imprinting of masculine behaviour [11]. The Leydig cells remain immature until puberty, when differentiation into mature Leydig cells occurs and testosterone production increases again, also stimulating Sertoli cell maturation (down-regulation of AMH) and spermatogenesis. An intriguing factor in testicular development is that, although testosterone concentrations are as high in the fetal and early postnatal period as in puberty, Sertoli cells remain immature and spermatogenesis is arrested until the onset of puberty. This is confirmed by the absence of spermatogonia in testes obtained by autopsy from boys aged 28 weeks of gestation to 4 years [12]. An explanation could be the lack of androgen receptor expression in fetal and neonatal Sertoli cells [13,14].

In females, due to the absence of a Y chromosome the Mullerian ducts develops into the female reproductive system. By 9 weeks of gestation the vagina begins to form, the Mullerian ducts fuse and the Wolffian system regresses [7]. Germ cells enter meiosis and by the 11th week clusters of oogonia are visible. Although gonadotropins are already formed in the pituitary by 10 weeks of gestation [9,15] and interstitial cells with steroid producing capacity are present, few, if any, steroids are actually produced by the developing ovaries. Aromatase activity is found in multiple tissues, but mRNA expression is low and the fetus is not capable of de novo estrogen production [16]. The increase in placental estrogen production, which is dependant on precursors produced by the mother and the fetus, promotes the development of follicles within the fetal ovary and seems to plays a role in programming events that are crucial for further development of the reproductive system [17]. At 18 weeks the first primordial follicles are detected [7]. Their number increases, reaches a peak around 5 months, decreases, and at term roughly 2 million oocytes remain [5]. Around seven months of gestation all of these germ cells have entered long stage meiotic prophase and remain quiescent until puberty [7].

Nowadays, we know that the onset of puberty is largely dependant on a substance called kisspeptin which is responsible for regulating higher brain regions resulting in activation

of the hypothalamic-pituitary-gonadal axis by stimulating GnRH secretion [18]. Although still under exploration, kisspeptin seems to play a role in the regulation of the fetal reproductive axis as well [19]. From early to mid-gestation, gonadotropin secretion does not seem to be influenced by kisspeptin activity. However, by the time the fetus reaches mid-gestation, the hypothalamic-pituitary-gonadal axis is fully functional and kisspeptin stimulated GnRH secretion results in pituitary gonadotropin production [20]. Between 30 weeks of gestation to full term a decline in kisspeptin activity co-incides with a decline in gonadotropin concentrations [19]. As GnRH neurons only exhibit estrogen-beta receptors, it is likely that gonadal feedback (by estrogens and androgens) also operates via kiss neurons which exhibit both androgen and estrogen-alpha receptors [21]. As mentioned earlier some of the hormones produced by the fetus are biologically inactive, however they might play a role in fetal programming. There is limited literature indicating a persisting epigenetic link between early life events and subsequent disease risk in humans [22]. Epigenetic effects include genetic imprinting which acts through DNA methylation and chromatin modifications [23]. For example, an increased risk of developing breast or testicular cancer in dizygotic twins was explained by intra-uterine exposure to high estrogen concentrations [24] (figure 1). Furthermore, the onset of autistic disorders [25], polycystic ovary syndrome [26], metabolic syndrome and cardiovascular disease [27] have all been suggested to be associated with hormonal changes during gestation. Not only physical but mental conditions have been reported, such as elevated testosterone concentrations and peri-partum depression [28] or more masculine play behaviour in girls [29].

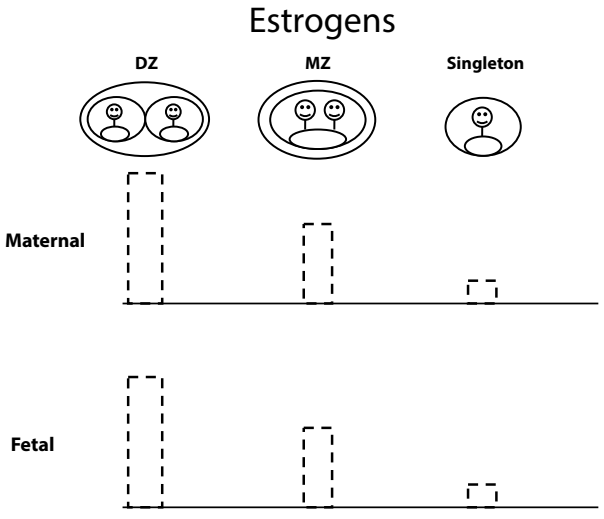

Figure 1. A visual representation, assumed in existing literature, of potential estrogen exposure/ influence in twin and singleton pregnancies and their offspring. DZ = dizygotic twin, MZ = monozygotic twin. During gestation and post-partum DZ twins are suggested to be exposed to higher estrogen concentrations compared to MZ twins and singletons.

Although much thought has been given to hormonal exposure during gestation we do not know much about the actual hormone concentrations affecting the developing fetus. For a pregnancy to sustain, an adequate interaction between the mother and the fetus has to develop. An example of the maternal-placental-fetal unit working as one is demonstrated in figure 2, which reports on the estrogen and progesterone synthesis during gestation. Estrogens in pregnancy enhance uptake of cholesterol which is important for placental steroid production. Although most of the estrogens are synthesized by the placenta, it is dependent on the production of DHEA(S) by the mother and the fetus [23]. As gestation reaches term most of the precursors needed are produced by the fetus, either in the adrenal (DHEAS) or in the liver (16 α-hydroxyl DHEAS) [23]. Progesterone is a steroid hormone which during early gestation is produced by the corpus luteum, but around 2-3 months the placenta takes over (luteal-placental shift). It stimulates the growth and development of blood vessels supporting the uterus and it decreases smooth muscle contractions [30]. Progesterone is produced in the trophoblast from pregnenolone and almost 90% is secreted into the maternal circulation.

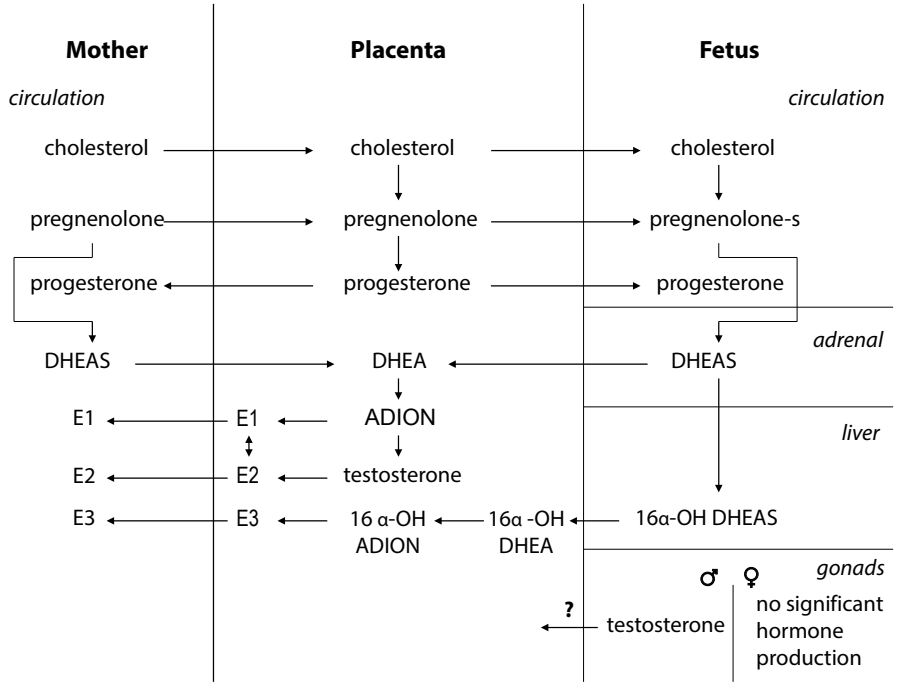

Figure 2. demonstrates the maternal-placental-fetal unit and the sex steroid synthesis during gestation [5].

As fetal blood sampling involves risks for the ongoing pregnancy substitutes such as maternal serum and amniotic fluid have been used to evaluate hormonal exposure during gestation. Poor correlations between maternal serum samples and umbilical cord blood have been reported [31]. In multiples it is even more complicated because circulating hormones both influence and are influenced by at least two fetuses. Although indirect, there seems to be evidence that androgens in opposite-sex twin pregnancies influence the female co-twin (figure 3). Despite the obvious importance of detailed information we have to deal with gaps of knowledge. An overview of hormonal concentrations during pregnancy in singletons and twins is lacking. Hormones may consist of different fractions, or active and inactive forms, and a variety of different assays is used to measure them, which makes it even harder to compare already published data. To make a more valid statement about the intra-uterine conditions influencing a developing fetus we need solid data for singletons and twins measured by the latest techniques and accounting for possible confounders such as zygosity, ethnicity and gestational age at birth.

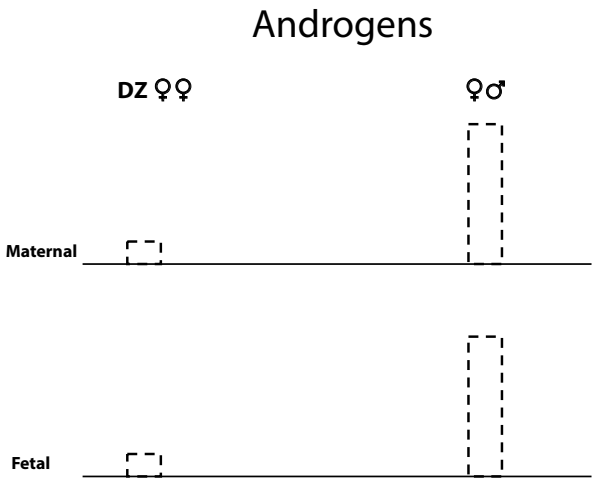

Figure 3. A visual representation, assumed in existing literature, on how girls of opposite-sex twins are influenced by their male co-twin. DZ ♀♀ = girl of a dizygotic girl-girl twin, ♀♂ = girl of an opposite-sex twin. Androgens during gestation and post-partum are suggested to be higher in girls who have a male co-twin compared to girls of DZ girl-girl twins, due to androgen production by the brother.

Outline of the thesis

In **chapter 2** we provide an overview of the existing literature on reproductive hormone concentrations in singleton and twin pregnancies. By conducting these data into figures we aimed to establish a rough estimate of hormonal changes that occur during gestation and in the first 6 months after birth. Remarkably, there was very limited information available especially for twin pregnancies. The main purpose of this thesis was to fill part of this gap of knowledge on perinatal reproductive endocrinology and evaluate suggested effects later in life. In order to do this we needed to select appropriate measuring techniques especially in neonates. **Chapters 3 and 4** describe the methods used for measuring gonadotropins in urine instead of serum and the normative data needed to interpret ultrasonographically measured testicular volumes. The results of a prospectively collected series of reproductive hormones, measured in maternal serum at mid-gestation and delivery (estrogens, androgens and progesterone) and in umbilical cord blood (estrogens, androgens, progesterone, gonadotropins, AMH and inhibins) are demonstrated in **chapter 5**. Hormonal profiles, gonadotropin levels in urine and testicular volumes were compared between singletons and twins and different types of twins. To evaluate suggested effects in adults we report on; 1) the prevalence of PCOS in opposite-sex twin girls compared to same-sex twin girls in **chapter 6**, and 2) the inhibin B and FSH feedback loop in male twins in **chapter 7**. In **chapter 8** we discuss the findings presented in this thesis and provide options for future research and **chapter 9** contains a summary of our work.

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