Development of the human fetal testis

Développement testicule fœtal chez l’homme

Peter J. O’Shaughnessy a,∗, Paul A. Fowler b

a College of medical, veterinary and life sciences, institute of biodiversity, animal health and comparative medicine, university of Glasgow, Bearsden road, Glasgow, G61 1QH, United Kingdom

b Division of applied medicine, institute of medical sciences, university of Aberdeen, Aberdeen, AB25 2ZD, United Kingdom

Abstract

Masculinisation and adult fertility in the male are dependent on appropriate fetal endocrine programming. There is also now increasing evidence to indicate that the same mechanisms which regulate masculinisation also affect the general wellbeing of males throughout their life and, particularly, during ageing. Testosterone, secreted by the fetal testes, is the main factor regulating these processes and an understanding of fetal testis development in the human male is essential if we are to prevent adult reproductive disorders. This review focuses on what is known about human testis development and describes the effects of maternal smoking, a surrogate of possible xenotoxicant exposure on fetal testis and fetal liver function.

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Keywords: Human; Fetal; Liver; Testis; Steroidogenesis; Maternal smoking

Résumé

La masculinisation et la fertilité de l’homme à l’âge adulte sont dépendantes d’une programmation fœtale endocrine appropriée. Il existe également maintenant des preuves croissantes que ces mêmes mécanismes qui régulent la masculinisation affectent aussi le bien-être général des sujets masculins tout au long de leur vie, y compris au cours du vieillissement. La testostérone, sécrétée par les testicules fœtaux, en constitue le principal facteur régulateur et la compréhension du développement du testicule fœtal chez le sujet masculin est essentielle à la prévention des troubles de la reproduction chez l’adulte. Cette revue est focalisée sur ce que l’on sait du développement du testicule fœtal. Elle décrit les conséquences du tabagisme maternel en tant que possible marqueur de l’exposition aux perturbateurs endocriniens sur le testicule fœtal et la fonction hépatique fœtale.

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Mots clés : Homme ; Fetus ; Foie ; Stéroïdogenèse ; Tabagisme maternel


∗ Corresponding author.

E-mail address: peter.oshaughnessy@glasgow.ac.uk (P.J. O’Shaughnessy).

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1. Introduction

Following the epidemiological studies of Barker et al. in the 1980s, it has become clear that adult health and wellbeing are critically dependent on the environment experienced during fetal development [1]. The key to understanding many adult disorders is likely to be dependent upon knowledge of how an individual’s adult phenotype is shaped during development and how these processes can be deregulated. In the male, masculinisation and adult fertility are dependent on appropriate fetal endocrine programming and there is now increasing evidence to indicate that the same mechanisms which regulate masculinisation also affect the general wellbeing of males throughout their life and, particularly, during ageing. Endocrine programming in the fetal male is mediated largely through testosterone secreted by the testes. Testosterone masculinises the fetal reproductive system by inducing growth and development of the Wolffian ducts, the accessory glands and male external genitalia. Disruption to this process will cause a spectrum of disorders ranging from pseudohermaphroditism to cryptorchidism and reduced adult sperm counts. An understanding of fetal testis development is a prerequisite, therefore, to understanding and preventing adult reproductive disorders. Animal models are invaluable for a mechanistic understanding of development but there are enough differences in testis development between humans and non-primate models to make it essential that we have a detailed knowledge and understanding of events in the human.

Testicular differentiation begins in the human at about 6 weeks after conception with the development of testicular cords containing Sertoli cells and gonocytes [2]. The Sertoli cells start secreting anti-Mullerian hormone (AMH) soon after differentiation, leading to the process of Mullerian duct regression, which is complete by mid gestation. Leydig cells can be seen in the interstitium of the fetal testis by week 8 [3,4] although the testes will show steroidogenic activity in vitro at 6–7 weeks after conception with the development of testicular cords towards the end of the first trimester (Fig. 1) [11]. If germ cell numbers are dependent on Sertoli cell number during fetal development, as they are in the adult, then germ cell proliferation may be limited by Sertoli cell numbers during the second trimester. Gonocyte migration to the basement membrane and differentiation to spermatogonial stem cells (SSC) and spermatogonia commences towards the end of the second trimester in humans and lasts for up to 8 months postnatally [16]. The process is gradual and asynchronous in primates, which means that while spermatogonia form in fetal life, undifferentiated germ cells at different stages of development will still be present throughout fetal and neonatal development. The differentiation step from gonocyte to SSC is particularly important as it has been reported that carcinoma in situ (CIS) cells arise from arrested/dysfunctional gonocytes [17].

2. Cell development

Combined analysis of three separate studies, all of which used the unbiased optical dissector technique, have given us a clear view of developmental changes in human testis cell number during the first and second trimester (Fig. 1). Our studies have shown that fetal Sertoli cell number in the human doubles approximately every 2 weeks from week 7 of gestation to week 19 [10,11]. The rate of proliferation drops during the third trimester and Sertoli cell number doubles three more times to reach about $130 \times 10^6$ cells/testis at birth [12]. Leydig cell numbers increase exponentially during the first half of the second trimester reaching a maximum number of about $2 \times 10^6$ at 18 weeks [11]. Thereafter, Leydig cell numbers decline in the human fetus up until birth through a process of dedifferentiation or degeneration [13,14]. Germ cell numbers show an exponential increase during fetal development from the start of testis differentiation until the end of the second trimester [11]. The rate of increase in germ cell numbers is greatest from 6 weeks until 10 weeks and is then reduced until the end of the second trimester [15]. The reason for this slowing of germ cell proliferation after 10 weeks is not clear but may be related to the number of Sertoli cells. There is a clear increase in the germ cell/Sertoli cell ratio between the first and second trimester, caused by the slower proliferation rate of the Sertoli cells during the first trimester (Fig. 1) [11]. If germ cell numbers are dependent on Sertoli cell number during fetal development, as they are in the adult, then germ cell proliferation may be limited by Sertoli cell numbers during the second trimester. Gonocyte migration to the basement membrane and differentiation to spermatogonial stem cells (SSC) and spermatogonia commences approximately every 2 weeks from week 7 of gestation [16]. The process is gradual and asynchronous in primates, which means that while spermatogonia form in fetal life, undifferentiated germ cells at different stages of development will still be present throughout fetal and neonatal development. The differentiation step from gonocyte to SSC is particularly important as it has been reported that carcinoma in situ (CIS) cells arise from arrested/dysfunctional gonocytes [17].

3. Control of development

In mammalian species for which we have data, initial development of the testis is independent of pituitary support. Thus, data available from the boar, rabbit, stallion, bull, ram, rat/mouse and dog would suggest that fetal Leydig cell function develops, at least initially, without a need for LH stimulation [18]. The hypothalamic-pituitary axis develops about halfway through gestation in most of these species and the testes become LH-dependent soon after [18]. In primates, including the human, the testes appear to go through an early phase of hormone-independence but, unlike other species, this is relatively short and the Leydig cells rapidly become dependent on the activity of chorionic gonadotrophin (CG) which acts to stimulate the LH-receptor (LHCG). This is clear from humans with an inactivating mutation in the LHCG or LHß subunit. Individuals who are XY but lack LHCG activity have an external female phenotype consistent with a lack of testicular androgen production during fetal development [19]. Remnants of some androgen-dependent structures (epididymis and ductus
deferens) are present, however, indicating a period of early Leydig cells activity in these individuals [19]. This is also supported by the observation that testes from 6–7 week old fetuses will maintain a steady secretion of testosterone in vitro without trophic support [5]. The fetal Leydig cells rapidly become dependent upon stimulation through LH/CR, however, and this support is clearly provided by CG rather than LH as fetal masculinisation is normal in individual lacking active pituitary-derived LH [20]. Levels of hCG peak at around week 10 [21] of pregnancy then decline and fetal androgens show a similar pattern which is consistent with a primary Leydig cell dependence on hCG [7]. Interestingly, the fetal Leydig cells appear to be relatively insensitive to hCG/LH stimulation since the LH+hCG/testosterone ratio is 14–22 in the male fetus but the LH/testosterone ratio is only 0.02–1.08 in the adult [7]. During the second trimester, as fetal androgens decline, LH may become more important for maintaining Leydig cell function since LH and hCG levels converge to a similar level by the end of the second trimester [7,22]. This is supported by observation of anencephalic human fetuses, which have an apparent loss of fetal Leydig cells in the second and third trimester and are often born with small external genitalia [23,24]. Since penis size is dependent upon androgen action beyond the MPW, this would suggest that Leydig cell function in late gestation is dependent on LH. Further evidence also comes from work in the rhesus monkey in which hypophysectomy during the final third of gestation leads to a loss of Leydig cells at term [25].

Sertoli cell proliferation and function post-natally are dependent on both FSH and androgen. During fetal development, it is likely that initial Sertoli cell activity is independent of hormonal stimulation as the hypothalamic-pituitary axis is not functional during early testis development. Later proliferation is clearly dependent on gonadotrophin support although, rodents apart [26], it is difficult to dissociate the effects of FSH from those of androgen. In anencephalic fetal humans, the testes are generally smaller, which is an indication that Sertoli cell number is probably reduced [23]. Similarly, treatment of fetal cynomolgus monkeys with GnRHa reduces testis size at birth [27] indicating that gonadotrophins are required for Sertoli cell proliferation.
rabbit and human use

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gestive that exposure to cigarette smoke may be associated

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ality, altered reproductive hormone levels, earlier puberty, reduced

testis size, reduced sperm counts (by 20–40%) and sperm qual-

development with increased incidence of hypospadias, reduced

unwanted effects on the offspring, many of which persist into

adulthood. These effects in males include altered reproductive

during fetal development and not in the fetal Leydig cell population,

Expression of HSD17B3 shows a similar pattern of development

although it is not clear whether this enzyme is predominantly

expressed in the Leydig cells during fetal development in

humans. In mice, HSD17B3 is expressed in the Sertoli cells dur-

ing fetal development and not in the fetal Leydig cell population,

although it is not known whether this is a general phenomenon

[32,33]. An alternative or “backdoor” pathway of androgen syn-

thesis has been described which generates the potent bioactive

androgen dihydrotestosterone without using testosterone as an

intermediate. This pathway was first described in 2003 in testes

from the tammar wallaby [34] and the relevance to human testic-

ular development and fetal masculinisation became clear when

mutations in AKR1C2 and AKR1C4, which encode critical

enzymes within the alternative pathway [35], were shown to be

linked to disordered sexual development (DSD). Since defects in

the canonical pathway will also lead to DSD, both pathways of

testicular androgen biosynthesis appear to be essential for nor-

mal human masculinisation. Clearly, developmental changes in

the balance between these pathways are an area that is in need

of further research.

5. Effects of maternal smoking on human fetal testis
and liver development

Maternal cigarette smoking during pregnancy has multiple

unwanted effects on the offspring, many of which persist into

adulthood. These effects in males include altered reproductive

development with increased incidence of hypospadias, reduced

testis size, reduced sperm counts (by 20–40%) and sperm qual-

ity, altered reproductive hormone levels, earlier puberty, reduced

final height and possible increased incidence of cryptorchidism

[9]. These effects on reproductive development are highly sug-

gestive that exposure to cigarette smoke may be associated

with deficient androgen production or action in utero but the

mechanisms by which maternal smoking affects reproduc-

tive development are unclear. Numbers of women who smoke

through pregnancy are decreasing but it remains at around 20% of

women in the United Kingdom with the rate significantly higher (up to 60%) in some social groups. This means that

maternal smoking per se remains the major, preventable cause of

developmental disorders and of considerable concern. The avail-

ability of fetuses from these women does mean, nevertheless,

that maternal smoking also offers one of the few mechanisms

by which we can directly study the effects of environmental pol-

lutants on human fetal health. Over 4800 chemicals are found in

tobacco smoke, and many of these are the same as the potential
toxicants found in the environment. These include heavy metals,
polycyclic aromatic hydrocarbons (PAH), volatile hydrocar-
bons, aldehydes, aromatic amines, and nitrosamines. Fetuses

from smoking mothers, therefore, are a unique model of tox-

ciant exposure that can be exploited to generate insight into the

general effects of pollutants/toxicants on human development,
especially where direct investigation of the fetus is impossible.

Below we describe the effects of maternal smoking on the fetal

testis and also the fetal human liver, as the liver may be the organ

most affected by xenotoxins derived from the mother.

5.1. Fetal liver

Protection of the fetus from xenotoxicants in smoke and from

environmental sources is through maternal metabolism, placen-
tal metabolism, and metabolism by the fetus itself (Fig. 2). In

the human, the liver starts to form in the fourth week of ges-
tation, and the basic structure is in place at the end of the first

trimester [36]. During gestation, the fetal liver receives 70% of

its blood supply from the umbilical vein and, therefore, directly

from the placenta and the feto-maternal interface. This means

that the fetal liver is exposed to the highest concentrations of

maternally-derived xenochrome. Unlike the rodent, the pri-

mate fetal liver is active during fetal development and is the most

important fetal organ for drug metabolism [37]. By the end of

Fig. 2. How exogenous chemicals reach the fetus. Maternal intake of chemicals,

including pollutants and drugs, occurs via multiple routes of exposure, with

maternal biotransformation (metabolism, modification, conjugation, sulphation)
in the gastro-intestinal tract, liver and placenta. The fetus will be exposed to

d xenochromes through the feto-maternal interface at the placenta. Fetal blood

from the placenta travels through the umbilical vein and supplies 70% of the

blood going to the fetal liver.
the first trimester, several enzymes involved in drug metabolism are already expressed in the fetal liver and there is evidence from primates that fetal levels of some metabolites exceed maternal levels [38]. The most important metabolic hepatic enzymes are the phase 1 and phase 2 enzymes. These act to detoxify xenobiotics through oxidation, reduction, and hydrolysis reactions (phase 1) and through conjugation reactions (phase 2), which normally inactivate the compound and increase excretion rates. Levels of PAHs in fetal human liver were significantly increased if mothers smoked and, of 37 transcripts associated with phase 1 and phase 2 metabolic reactions, 17 (46%) were significantly affected by maternal smoking, of which 80% were induced [39]. Surprisingly, sex differences in enzyme expression were also seen with the effects of smoking predominantly seen in males, which may affect levels of endogenous factors involved in fetal growth [39]. This data shows that, as a model, the smoke-exposed fetus is likely to be of significant use in identifying xenotoxin-dependent mechanisms of developmental deregulation.

5.2. Fetal testis

Maternal smoking during the second trimester significantly reduced fetal hCG levels by 40% (103 U/L vs 64 U/L. \( P = 0.021 \)) [7]. Testosterone levels were slightly, but not significantly, reduced by maternal smoking but the reduction in hCG may leave the fetus more susceptible to other perturbations which affect Leydig cell function. Maternal smoking has been reported to reduce germ cell and somatic cell numbers in the fetal testis during the first trimester [40] (and to reduce fetal ovarian germ cell proliferation [41]), although we did not see any differences in Leydig, Sertoli or germ cell numbers in a different cohort during the second trimester [42]. It is not clear, comparing these studies, whether the germ cell number rebounded to normal during the second trimester but as the same unbiased cell-counting techniques were used in the two studies, this would appear likely [15]. To identify possible targets of maternal smoking in the fetal testis, we have measured expression of 30 different transcripts during the second trimester [42]. Of these transcripts, only one, desert hedgehog (DHH), was significantly reduced by maternal smoking. DHH is secreted by the Sertoli cells and is a critical regulator of testis development in rats, mice and humans [43,44]. It remains to be determined, however, whether and how a reduction in fetal DHH levels would affect testis development in utero and post-natally. Whether maternal smoking affects other developmental pathways in the testis is unknown and awaits global analysis of transcript expression in the different cohorts.

6. Conclusions

By the nature of the subject, studies on the human fetal testis are largely confined to what can be observed or measured directly, along with insights that can be gained from the phenotype of natural mutations. In general, however, the pattern of human testis development follows that of other species but with some characteristic features such as:

- the human has an absolute requirement for LHCGR activation from early on in the process;
- the endocrine milieu of the developing human fetus differs significantly from other species although there is a clear need for more data from direct measurements of fetal plasma [45];
- androgen synthesis pathways in the fetal human testis are considerably more complex than once thought although whether this is unique to the human remains to be determined.

It is likely that more comprehensive and detailed study of the effects of maternal smoking, including for example epigenetic changes, will identify mechanisms by which xenotoxicants can affect fetal development and the testes in particular. The recent development of testis explant systems will also allow systematic study of the effects of potential harmful agents [46].

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

References


