From THE DEPARTMENT OF LABORATORY MEDICINE
DIVISION OF CLINICAL PHARMACOLOGY
Karolinska Institutet, Stockholm, Sweden

STEROID METABOLISM IN HUMAN REPRODUCTIVE ORGANS

Anna Helena Karypidis

Karolinska Institutet

Stockholm 2011

All previously published papers were reproduced with permission from the publisher.

Published by Karolinska Institutet.

© Anna Helena Karypidis, 2011

ISBN 978-91-7457-169-1

Printed by
REPROPRINT AB
Stockholm 2011
www.reproprint.se
Gårdsvägen 4, 169 70 Solna

Det första hon gjorde var att halka på det isiga berget och sätta sig mycket hårt.
Jasså, sa lilla My hotfullt. Står det till på det viset!
Så kom hon att tänka på hur en My ser ut med benen i vädret och skrattade länge för sig själv.

Ur Trollvinter, Tove Jansson

ABSTRACT

Androgens are involved in the development of prostate cancer. Both UGT2B17 and CYP7B1 are involved in the metabolism of androgens and are highly abundant in the prostate.

Deletion of the UGT2B17 gene is associated with low or undetectable urinary testosterone levels. The phenotypic outcome of the deletion was studied by quantifying the UGT2B17 mRNA expression in normal prostate tissues in individuals with different genotypes. Additionally a case–control study of prostate cancer was performed, including 176 cases diagnosed with prostate cancer and 161 healthy controls. Individuals homozygous for the insertion allele expressed 30 times higher levels of UGT2B17 mRNA in prostate tissue compared to heterozygous carriers, who had a significantly increased risk of prostate cancer (OR 2.15, CI 1.29–3.58).

We screened the human CYP7B1 gene for possible polymorphisms. Only one single polymorphism was detected, a C–G change in the promoter. Expression studies with reporter constructs showed significantly higher transcriptional activity of the G variant in Hek293 cells (2.7-fold) The allele frequency was 4.04% in Swedes and 0.33% among Koreans. No association to prostate cancer could be found when tested in the previously described case–control study.

In a population-based case-control study including 507 women with miscarriage in the first trimester of pregnancy and 908 controls with a normal first-trimester pregnancy, it was determined whether the cytochrome P450 1B1 (CYP1B1) Val432Leu polymorphism is associated with risk of miscarriage. Carriers of the Val/Val genotype were at higher risk of miscarriage in the first trimester of pregnancy compared to Leu/Leu carriers (OR 1.46; 95% CI 1.02–2.08).

When the same single nucleotide polymorphism (SNP) was investigated in a case–control study of recurrent miscarriages, 206 women who had had three or more miscarriages were compared to 618 controls who had at least one pregnancy and who never miscarried. The OR for Val/Val genotype carriers was 1.00 (95 % CI 0.64–1.56), giving no association with repeated miscarriage. Thyroid disease and smoking was significantly associated with recurrent miscarriages.

The association between abnormal progression of the first stage of labour and expression of enzymes involved in the androgen metabolism as well as estrogen receptors alpha and beta in human myometrium was investigated. Twenty women with an abnormal cervical ripening were compared to 12 women with a normal progression of cervical dilatation and 15 women that had not been in labour. Estrogen receptor alpha had a significant role in the progression of human labour. Estrogen receptor beta, AKR 1C1-4, CYP 19A1, CYP7B1 and 5alpha reductase type 1 expression in the myometrium are independent of the progression in the first stage of labour.

LIST OF PUBLICATIONS

I. KARYPIDIS, A. H., Olsson, M., Andersson, S. O., Rane , A.,Ekstrom, L.
Deletion polymorphism of the UGT2B17 gene is associated with increased risk for prostate cancer and correlated to gene expression in the prostate
Pharmacogenomics J, 2008, 8, 2, 147-51

II. Jakobsson, J.,KARYPIDIS, H.,Johansson, J. E.,Roh H. K.,Rane, A.,Ekstrom, L.
A functional C-G polymorphism in the CYP7B1 promoter region and its different distribution in Orientals and Caucasians
Pharmacogenomics J, 2004,vol 4,issue 4, p 245-50

III. KARYPIDIS A. H.,Soderstrom T.,Nordmark A.,Granath F.,Cnattingius S., Rane A. Association of cytochrome P450 1B1 polymorphism with first-trimester miscarriage
Fertility and Sterility Volume 86, Issue 5, November 2006, Pages 1498-1503

IV. KARYPIDIS A. H., Sundström Poromaa I, Hosseini, F, Skjöldebrand-Sparre L, Rane A, Bremme K, Stavreus-Evers A, Landgren B-M
Cytochrome P450 1B1 polymorphism is not associated with recurrent miscarriage
Manuscript

V. KARYPIDIS A H, Stavreus-Evers A, Wiberg-Itzel E, Ekström L, Rane A, Landgren B-M, Åkerud H
Estrogen receptor alpha expression decresed in dystocia; a clinical study on mRNA expression of sex steroid related enzymes during parturition
Manuscript

CONTENTS

LIST OF ABBREVIATIONS

AMH	anti-Müllerian hormone
5AR1	5-alpha reductase type 1
CI	confidence interval
CYP	cytochrome P450
DHA	dehydroepiandrosterone
DHT	dihydrotestosterone
ER	estrogen receptor
HCG	human chorionic gonadotropin
Leu	leucine
LDL	low density lipoprotein
OR	odds ratio
PCO	polycystic ovary syndrome
PCR	polymerase chain reaction
PSA	prostate specific antigen
SNP	single nucleotide polymorphism
SRY	sex-determining region on the Y chromosome
T	testosterone
UGT	uridine diphosphoglucuronosyl tranferase
Val	valine

1 INTRODUCTION

1.1 GENERAL INTRODUCTION

Reproductive steroid hormones are divided into three major groups; estrogens, progestins and androgens. These are of great importance in the sexual development and health of men and women, and are interact with all organs in the body including the brain.

Androgen is a Greek word derived from ανδρας (man) and γεννω (giving birth). Androgens are steroids mainly metabolised by the testicles, which are necessary for the development of gender and primary and secondary sex characteristics of the male [1]. Androgens have been in focus for years as hormones that can influence the development and progression of prostate cancer, the most common form of cancer in men (34.1 % of all male cancers in Sweden 2007 ref SOS Cancer i siffror 2009).

Androgens (e.g. testosterone and dihydrotestosterone) are also formed in the ovaries. However, the mean total levels of testosterone in women are only 1/10 of those in men [2]. The function and effects of these hormones, nevertheless, also are of importance in women, an example is the androgenic contribution to PCO (polycystic ovary syndrome). For both men and women, lower than normal levels of circulating androgens, have been associated with sexual dysfunction although the exact mechanisms have not been established [3, 4].

Estrogens (e.g. estrone, estrone sulphate, estradiol, estriol) are mostly associated with sexual development of the female since they are required for the normal maturation of female primary and secondary sex characteristics during adolescence and for the onset of fertility in women. During fertile life, estrogens are mostly produced by the growing follicle, in the ovary. In pregnancy, the placenta is the main steroid producing organ, introducing very large amounts of circulating estriol and progesterone

[5]. Later in life estrogen levels will naturally decline, with post-menopausal values falling below those measured in the male (figure 1).

Target organs for estrogens are mainly the uterus and the breast and for testosterones the prostate and hair follicles.

1.2 SEX DIFFERENTIATION

The mammalian embryo is initially formed by precursor tissues that are the same irrespective of chromosomal sex. The precursor tissue is the indifferent gonad, which will be differentiated to testis or ovary depending on the existence of a Y chromosome. The Müllerian and Wolffian duct systems are the respective precursor tissue for the female and male internal genitalia, and the urogenital sinus which gives rise to external genitalia [6].

Sexual development in the mammalian embryo depends on three sequential processes. The first step, which occurs at fertilisation, is to establish the genetic sex by the content of the sex chromosomes. Gender is determined by the presence or absence of a Y chromosome. When present, the Y chromosome dominates over the X chromosome (even if multiple), leading to male sex differentiation [7].

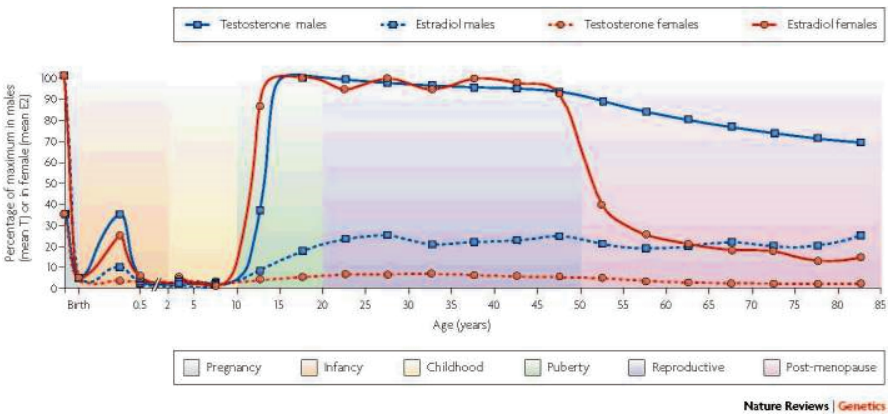
The Y chromosome will be the factor that leads to the second step of development of the male, the differentiation of the indifferent gonad to testis. Experimental studies have shown that ovarian differentiation is the "default pathway" in mammals [6]. In males, this pathway is "overridden" by the testis determining gene which is located on the Y chromosome (SRY) [8]. It seems that SRY acts on a single gene *SOX9*, which then drives the Sertoli cell formation giving rise finally to the testis. In the female *SOX 9* is down-regulated. It has been thought that this down-regulation is passive, however a hypothesis that has been questioned.

In the third step, the final gonadal phenotype arises depending on the presence (male) or absence (female) of three hormones, the anti Müllerian hormone (AMH), testosterone (T) and dihydrotestosterone (DHT). In the male, the foetal testis secretes AMH that affects the Müllerian ducts, which otherwise (in the female) are precursors of the fallopian tube, uterus and the upper part of vagina [9]. Separately, testosterone

secretion from the testis drives growth and differentiation of the Wolffian duct system, giving rise to male internal genitalia, epididymis, vas deferens and seminal vesicles [10]. The external male genitalia (prostate, scrotum, penis and male urethra) will develop when the urogenital sinus is affected by DHT, the 5-alpha reduced metabolite of testosterone [11]. As noted, female development ensues in the absence of these androgenic steroids, otherwise derived from the foetal testis. The preeminent role of these hormones in male development has been proved by experimental studies showing that the female foetus can be masculinised if androgens are provided even if testis are missing, as in the androgenital syndrome[5, 12].

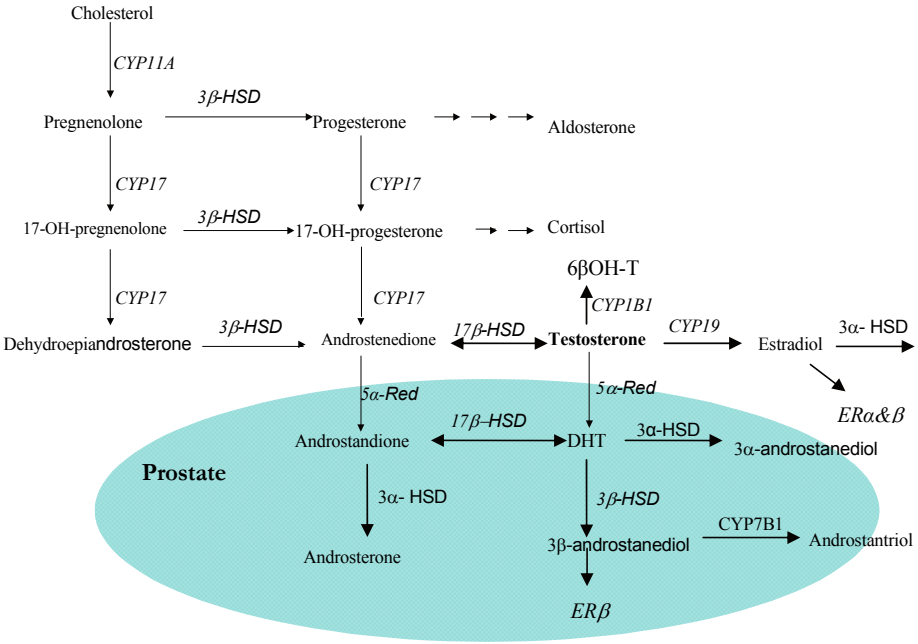
Although the expression of androgens in the embryonic stage, are closely related to normal male development, androgens have been found in females throughout life. (fig 1)

Figure 1



Approximate mean sex steroid levels in plasma in males and females
Variation in steroid levels is shown as percent of the maximum mean testosterone (T) in males and the maximum mean estradiol (E) in females across the life stages.
Published in final edited form as:
Nat Rev Genet. 2008 December; 9(12): 911–922. [13]

1.3 STEROID METABOLISING ENZYMES



1.4 PROSTATE CANCER

Prostate cancer is the most common cancer form in man (comprising34.1% of all male cancers in Sweden 2007 ref SOS Cancer i siffror 2009). The clinical focus in recent years has been on early diagnosis of prostate cancer, with emphasis on general screening with PSA (prostate specific antigen). Several studies would appear to support the utility of the PSA screening. For example, a large European study on PSA testing found a 20% decline in prostate cancer related mortality among those men that were screened with PSA [14]. Further evidence was provided in a recent Swedish publication on PSA screening showing as much as a 50 % decrease in prostate cancer related mortality with PSA screening[15].

Enthusiasm for PSA screening programs, however, has been tempered by one major drawback, of PSA screening programs - the high percentage of overtreatment that is required to achieve the benefits of reduced mortality. Thus, it was estimated that to prevent one prostate cancer related death, 48 men had to be treated [14]. Since the treatment for localised cancer is prostatectomy or brachytherapy, adverse effects of impotence and incontinence have to be considered [16, 17].

The incidence and the relative 10 year survival rates in prostate cancer in the Nordic countries have been increasing substantially since 1964, probably an effect of the introduction of PSA testing [18] . However, the corresponding mortality in prostate cancer has been stable.

It is not surprising, then, that treatment of localised prostate cancer has been a matter of intense discussion and debate, which is further energized by the fact that the risk of dying due to localised prostate cancer is rather low (3.6 % 10-year mortality without treatment) [19]. When treatment is given to all prostate cancer patients, the mortality rate due to the disease is lowered by 34 % but not the overall mortality when compared to “watchful waiting” [20].

The importance of androgens in the development and progression of prostate cancer is widely known. It had been observed, for example, that eunuchs do not develop prostate cancer. In 1941, Huggins [21] reported that orchidectomy is an effective way to ameliorate the disease symptoms and to delay progression of advanced prostate cancer. The mechanisms underlying the androgenic influences on the etiology of prostate cancer are not well-understood. This is illustrated by a study in which men were given finasteride, a drug that is inhibiting the biotransformation of testosterone to the more potent metabolite dihydrotestosterone (DHT). This treatment reduced the incidence of prostate cancer overall, but was also found to accelerate the progression of more aggressive forms of prostate cancer. [22]

In order to improve the prognosis, without overtreatment, new diagnostic indicators are searched for such as gene polymorphisms affecting the metabolism of androgens.

1.5 PREGNANCY

In pregnancy, the foetus and mother are interrelated by signalling mostly through the hormonal environment. The growing foetus can influence or control its own growth by signalling to the mother. It has proven useful to consider the sex steroid metabolism in the foetus, mother and placenta as a unit, where each component is seen as contributing to the metabolism of the others. This concept of the "fetoplacental unit" was introduced by Egon Diczfalusy in 1964[23].

Progesterone, produced by the corpus luteum until the 10th week of gestation, is believed to be important for the maintenance of human pregnancy. Thereafter, the placenta becomes the predominant source for the production of large amounts of hormones (including progesterone and estrogens). The steroid-hormone precursor cholesterol must be provided by the maternal circulation, given that no cholesterol is produced within the placenta itself.

The uptake of cholesterol in the placenta is regulated by estradiol, which is found to increase the LDL-receptor gene transcription in baboons [24]. Estradiol is also enhancing the effect of P450scc (encoded by the CYP11A1 gene) that is the enzyme metabolising cholesterol to pregnenolone, the substrate from which progesterone is produced [25].

The androgens are the basic precursors of estradiol. In early pregnancy, they are provided from the maternal circulation. Later in pregnancy, the estrogen production is under control of the foetus, thus dehydroepiandrosterone sulphate (DHAS) is mostly produced in the foetus, before being converted in the placenta to testosterone, which then is aromatised to estradiol. Finally, estradiol will be secreted into the maternal circulation.

1.5.1 Miscarriage AND Repeated miscarriage

Ten to twenty percent of all clinically recognized pregnancies end in miscarriage [26] [27]. "Recurrent miscarriage," codified as 3 or more consecutive miscarriages, is experienced by 1-2 % of couples. [28].

The prevalence of recurrent miscarriage is higher than expected [28], suggesting common explanatory factors for the miscarriages.

It has been estimated that in over half of miscarriages, a chromosomal abnormality is present in the foetus [29] [30, 31]. Major parental chromosomal disorders such as balanced translocations explain 3-6% of miscarriages [32, 33].

Maternal age > 35 years is a major factor for miscarriage, largely through the clear association between maternal age and risk of chromosomal abnormality in the foetus [31, 34, 35].

Autoimmune factors (such as lupus anticoagulant and cardiolipin antibodies) also are well known to increase the risk of miscarriage [36]. The well-established treatment of women with known antiphospholipid syndrome and recurrent miscarriage with aspirin and fractionate heparin appears to lower miscarriage rates at least in early pregnancy[37, 38].

Thrombophilia is a diverse group of coagulation disorders associated with a predisposition to thrombosis. Except for antiphospholipid syndrome other conditions included are activated protein C (APC) resistance inherited through a mutation in factor V Leiden, deficiency of protein C and S, mutation in the prothrombin gene and antithrombin deficieny [39]. Although these conditions give an increased risk for thrombosis, there was no decreased risk found for miscarriage when aspirin and fractionate heparin was given to these women [40].

In a Cochrane metaanalysis in 2009 studying low dose aspirin treatment in women with repeated miscarriage but no trombophilia, showed no benefit of

anticoagulant treatment either. [41]. In conclusion, only women with known antiphospholipid syndrome will benefit from anticoagulant treatment.

Maternal exposure to antidepressants is another variable that has been linked to elevated risk for miscarriage [42]. It is not known, however, whether the association is better explained by the drug treatment or by factors related to the underlying disease. The mood of the pregnant women, more generally, is taken to be of relevance to pregnancy outcomes. For example, the practice of "love and tender care" has been shown to reduce the recurrence of miscarriages [43].

The importance of progesterone produced in the ovary during early pregnancy has lead to trials with corpus luteum-enhancing treatments, where HCG (human chorionic gonadotropin) injections or progestines are given, for prevention of miscarriage. Cochran meta analysis of these treatments studies, however, do not affirm consistent or reliable effects on pregnancy outcomes. Thus, neither progestagens nor HCG is recommended for the treatment of repeated miscarriages [44, 45].

It has been postulated that immunologic aberrations may be responsible for recurrent miscarriage. The physiological mechanisms that allow a mother to tolerate her semi-allergenic baby are unclear. Defects in molecular immunosuppressive factors at the local decidual/trophoblast level have been implicated [46]. Therefore, there have been several attempts to modulate the immunological response in the mother. These treatments were evaluated in a Cochrane review, but no effect on risk of miscarriage was found [47].

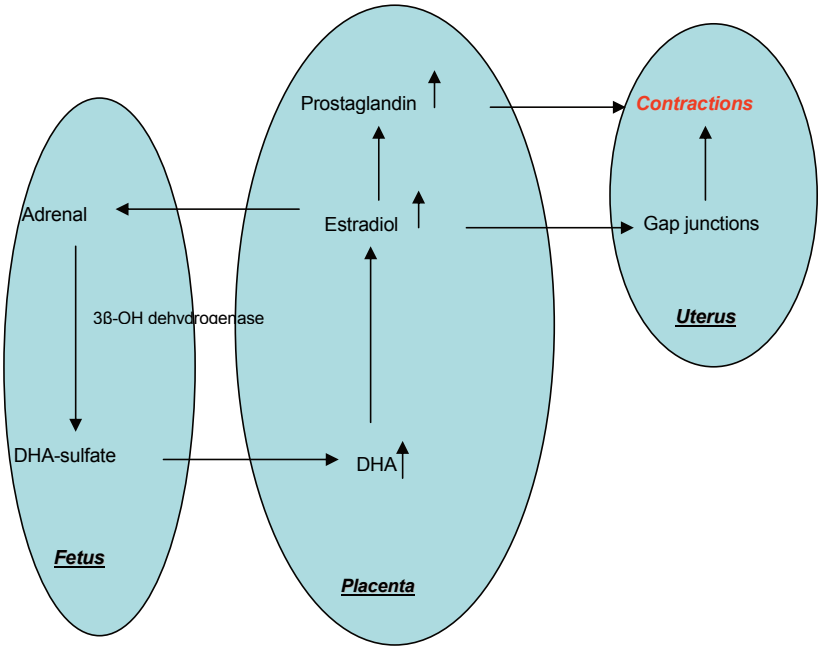


Figure 3
A model of induction of labour in sheep showing the interaction in the fetoplacental unit that leads to labour. (Speroff 2005)

1.5.2 Dysfunctional labour

The mechanism of initiation and progression of labour are still not known. The steroidal hormones, progesterones and estrogens are increasing during pregnancy. A rise in estradiol and decrease of progesterone are known to initiate labour in other species than humans [48]. However in studies measuring hormonal levels in women prior to the onset of labour, a similar alteration in steroidal levels in peripheral blood, could not be shown [49].

Estrogens are important for human labour since in pregnancies with low estrogen levels, as in anencephalia of the foetus, labour will not start in time. Progesterone administration on the other hand might prevent preterm delivery in high-risk singleton pregnancies [50]. This effect does not seem to be related to initial progesterone levels.

The increase of salivary estriol and the estriol/progesterone ratio throughout gestation seem to be of importance. The estriol/progesterone ratio rises even more dramatically immediately before term as well as in preterm labour [51]. In addition, two consecutively elevated salivary estriol measurements have been strongly associated with impending preterm delivery [52].

It has also been shown that the onset of labour in humans is related to relative progesterone withdrawal due to an increase in progesterone receptors in the myometrium [53] with a corresponding up-regulation of the estrogen receptor alpha leading to a higher responsiveness of the myometrium to estrogen prior to labour. [54]

Estradiol has also been shown to successfully induce labour, an effect comparable to that shown for prostaglandins that are dominating clinical practice today [55] [56] [57] [58]. High estradiol levels, in the myometrium, are believed to increase the local production of prostaglandins.

The most common indication for caesarean section among nulliparous women is dystocia or abnormal labour. Dystocia is characterised by absence of a successful progression of labour that can be due to; fetomaternal disproportion, unfavourable presentation of the foetus during birth and arrest in the progression of labour related to the dysfunction of the uterus and cervical ripening [59].

The standard treatment for dystocia when a lack of effective contractions of the uterus is suspected is the usage of intravenous oxytocin. Although oxytocin is widely used, the number of women undergoing caesarean section is steadily increasing in the western world. At the same time the adverse effects of oxytocin has been in focus for

research and active management of labour that includes an early usage of oxytocin has been questioned[60]. A more recent meta-analysis has shown only a modest effect, if any at all, of the usage of early oxytocin treatment (along with early amniotomy) on the caesarean section rate [61]. Therefore, more knowledge about the mechanisms of labour is needed.

2 THE PRESENT STUDY

2.1 AIMS

The aim of this thesis was to investigate possible effects of genetic variation of androgen and estrogen metabolism on human reproduction and reproductive organs.

The specific aims for each study were as follows:

Paper 1: To investigate the impact of the UGT2B17 deletion polymorphism on prostate cancer risk and further to investigate the effect of the deletion on the expression of UGT2B17 in the prostate.

Paper 2: To investigate the occurrence of single nucleotide polymorphisms in the CYP7B1 gene and to analyse possible effects of a novel polymorphism identified in the CYP7B1 gene on the risk of prostate cancer.

Paper 3: To investigate the effect of the CYP1B1 Val432Leu single nucleotide polymorphism (SNP) on the risk of miscarriage in the first trimester.

Paper 4: To determine whether the risk allele found in study 3 also is a risk factor for repeated miscarriage and, further, to describe the clinical characteristics of a population of women with repeated miscarriages.

Paper 5: To determine whether expression of different androgen metabolising enzymes and estrogen receptors is affected in the myometrium of women who have had a dysfunctional labour.

2.2 METHODS

2.2.1 Subjects

2.2.1.1 Papers 1-2

One hundred seventy six men, age 51–79 years, with known prostate cancer, were recruited consecutively in Örebro County Sweden. One hundred sixty one men were randomly selected from the county population register and matched for age (50–59, 60–69 and 70–79 years) served as control group.

In paper 1, a Korean population of 156 healthy men and women were used for comparison with the Swedish controls.

2.2.1.2 Paper 3

Four hundred ninety-eight women were recruited when diagnosed with miscarriage in the first trimester of pregnancy (6–12 completed weeks of gestation) from Akademiska University hospital. Nine hundred twenty-nine control subjects were recruited from the maternal care centers in the same catchment area.

2.2.1.3 Paper 4

Two hundred twelve women with three or more verified consecutive miscarriages in the first or second trimester of pregnancy (5-21 completed weeks of gestation) were recruited as cases. Six hundred thirty-six control subjects were randomly chosen from the Uppsala University biobank of pregnant women. Controls had at least one full term pregnancy and no history of miscarriage.

2.2.1.4 Paper 5

Three groups of women were included in the study: 1) 15 women planned for caesarean section before start of labour 2) 12 women with a normal progression of cervical dilatation until the labour ended in an emergency caesarean section and 3) 20 women with an abnormal progression of cervical dilatation that ended with an emergency cesarean section.

2.2.2 Laboratory methods

2.2.2.1 Genotyping

DNA was extracted from blood or buffy coat using a commercial kit and stored at –20˚ until analyzed.

A fluorogenic 5' nuclease polymerase chain reaction (PCR) was performed according to the principle reviewed by Livak[62], with a primer pair and two different probes identifying the two alleles. (Papers 1,3 and 4)

In paper 2, two primer pairs corresponding to the gene (*ins*) or to the area were the deletion is included (*del*) were used for the PCR. The products were identified either on a gel or by real time PCR (Taqman).

2.2.2.2 Single Nuclotide Polymorphism Detection

2.2.2.2.1 <u>In silico analysis</u>

Potential polymorphisms in the CYP7B1 gene were identified using the EST database and a BLAST alignment tool. When the indicated mutations gave rise to a

nonconservative amino-acid change, we performed a PCR-direct sequence analysis to verify these potential polymorphisms. (Paper 2)

2.2.2.2.2 Single-stranded Conformation Polymorphism Analysis

Single stranded PCR products of the gene were separated on polyacrylamide gels. The DNA fragments were visualized by silver staining. DNA fragments that have sequence differences will be separated on the gel. These fragments are then purified using PCR purification kit and sequenced. (Paper 2)

2.2.2.3 Reporter Gene assay

Plasmids were constructed by PCR amplification using human genomic DNA as template with the forward and reverse primers including the part of DNA of interest. The amplification products were subcloned into a pCR2.1 vector, and then subcloned into a luciferase vector. HepG2 (liver) and Hek293 (kidney) cells were transfected with the luciferase vector containing the DNA sequence of interest. The cells were incubated and luciferase activity was determined using a luminometer. (Paper 2)

2.2.2.4 Gene expression

Total RNA was extracted from the myometrial and prostate samples. The purity and quality of myometrial RNA was evaluated in an Agilent analyser. Synthesis of complementary DNA (cDNA) from RNA samples was performed using a commercial kit.

Relative quantification of cDNA was done using real time PCR (Taqman). The gene expression was normalized against an endogenous housekeeping gene. (Papers 1 and 5)

.

2.2.3 Statistical methods

Genotype and allele associations were assessed with χ^2-test, binary and nominally logistic regression. ORs were used as an approximation of relative risks, using 95% confidence intervals (Papers 1-4).

The statistical analysis for expression mRNA was performed using t-test for independent samples (paper 5) and the non parametric Mann-Whitney U-test (Papers 1 and 5). A p-level of 0.05 was considered as significant.

2.3 RESULTS

2.3.1 Papers 1 and 2

Individuals lacking one or two copies of the insertion (*ins*) (*del/del* or *del/ins*) of the UGT2B17 gene were at significantly increased risk of prostate cancer (OR 2.05) (table 1). The expression of UGT2B17 was found to be significantly lower in prostate tissues derived from individuals with deletion of one of the alleles as compared to that of *ins/ins* individuals (P <0.02, Mann–Whitney U-test). The *ins/ins* individuals exhibited approximately 30 times more UGT2B17 mRNA in the prostate tissues than individuals with only one *ins* allele (*ins/del*).

A functional SNP in the promoter region of the CYP7B1 gene was reported, otherwise the gene was highly conserved. The SNP was more frequent among Koreans than Swedes (p = 0.002). No association with prostate cancer risk could be demonstrated.

2.3.1.1 Table 1

Odds ratios for having prostate cancer for UGT2B17 del/ins and del/del variants compared with ins/ins carriers

		OR (adjusted)	**CI**
UGT2B17	**ins/ ins**	1	**Reference**
	ins/del	2.15	1.29–3.58

2.3.2 Papers 3 and 4

Relative to carriers of the Leu/Leu genotype, we found that carriers of the Val/Val genotype were at increased risk of first trimester miscarriage (OR 1.46).

Adjustments for covariates (maternal age, daily smoking, caffeine intake, earlier miscarriage, alcohol intake and pregnancy symptoms) did not essentially change this association (table 2).

The unadjusted odds ratio for recurrent miscarriages among Val/Val genotype carriers relative to carriers of the Leu/Leu genotype was 1.00 (table 2).

Independent explanatory factors for recurrent miscarriage in this population were thyroid disorders, other disorder with known association with recurrent miscarriage and smoking (Paper 4).

2.3.2.1 Table 2

Odds ratios for miscarriage and repeated miscarriage for carriers of the CYP1B1 Val/Val and Val/Leu variants compared with Leu/Leu carriers.

		OR (adjusted)	CI
Miscarriage	**Leu/Leu**	1	Reference
(Paper 3)	**Val/Val**	1.46	1.02–2.08
Repeated miscarriage	**Leu/Leu**	1	
(Paper 4)	**Val/Val**	1	0.64 – 1.56

2.3.3 Paper 5

Uterine estrogen receptor alpha (ERα) was up-regulated in the women with normal progression of labour compared to those with abnormal progression ($p = 0.008$) and to those not in labour ($p = 0.003$). The expression of ERβ, 5AR1, AKR1C1 and 3, CYP19A1 and CYP7B1 did not differ in the group of women with abnormal labour compared to the two control groups of women.

3 GENERAL DISCUSSION

The aim of this thesis was to investigate possible effects of genetic variation of androgen and estrogen metabolism on human reproduction and reproductive organs.

CYP7B1, together with 3a-HSD, 3b-HSD, 5 alpha-reductase as well as UGT2B17 gene are enzymes known to determine the levels of androgens and their metabolites. Genetic variations in these genes are important to study, in order to understand the mechanisms that promote the development and growth of prostate cancer. Identifying risk genotypes may also be important for physicians, when choosing therapy in order to offer the treatment with the best prognosis in combination with the lowest possible side effects.

This study has shown that individuals with two copies of UGT2B17 gene have significantly lower risk of developing prostate cancer compared to carriers of only one copy. (Paper 1) Additionally, it was found that the deletion of one allele is associated with significantly lower mRNA levels in the prostate, further supporting the epidemiological results that individuals exhibiting two gene copies of UGT2B17 are protected from androgen exposure in the prostate and thus have a decreased risk of developing prostate cancer. Our results are consistent with a previous report, which showed that Caucasian individuals, homozygous for the deletion allele exhibit a significantly increased risk for prostate cancer compared with insertion carriers (*ins/ins* and *ins/del*)[63]. The genotyping method used in that study did not distinguish between heterozygous (*ins/del*) and homozygous (*ins/ins*) genotypes, thus they were not able to compare the *del* carriers with individuals homozygous for the *ins* allele, as in this report. A later study, that also used a genotyping method that did not distinguish *ins* homozygous carriers from heterozygous carriers, concluded that no difference in risk exists to *del/del* carriers [64]. They used however, a reference group that included high

risk *(ins/del*) mixed with low risk individuals (*ins/ins*), thus no difference was found. In a study by Gallagher [65] no difference was observed, however they have been criticized for including individuals with deviating PSA levels in the control group. The risk of including individuals with prostate cancer in the control group is bias versus null [66].

A novel functional SNP in the CYP7B1 was identified (Paper 2). We observed differences between the mutant variants in promoter activity in Hek293 cells, whereas no enhancement of activity was observed with HepG2 cells. The difference could be explained by variation in the abundance of C/EBPb and/ or other proteins in the HepG2 and Hek293 cells. When the allele frequency of this SNP was analysed a difference was seen between Swedes and Koreans, which did not have an effect on prostate cancer risk in the Swedish population. This may be explained by lack of power, on the other hand the mutant allele was not common in the Swedish population giving a low impact in this population.

One limitation of these studies is small sample size, which could explain why we could not find any association with tumour stage or differentiation grade for the UGT2B17 deletion. However, the strengths of the study are that the healthy controls were age matched and recruited from the same community as the cases.

The CYP1B1 polymorphism was studied in papers 3 and 4. A common polymorphism that was earlier found to affect steroid metabolism was associated with first trimester miscarriage and was modifying the effect of coffee drinking. This result was however not confirmed in a case-control study on repeated miscarriages (Paper 4). The results are in line with Saijo and colleagues who did not find any association between this polymorphism and risk of two or more miscarriages in Japanese women [67]. In conclusion, the "risk polymorphism" might be associated with miscarriage; however, it is not an absolute obstacle for maintenance of a pregnancy.

As physicians, finding a simple explanation, for the diseases that strikes our patients would be fantastic! Miscarriage is a condition that has a multifactorial cause. The purpose of research concerning miscarriages is not to prevent every miscarriage but to increase the knowledge of how human pregnancy is thriving and which factors may affect a normal progression of pregnancy. Miscarriage is Natures way of selecting healthy pregnancies. Women that have had repeated miscarriages are at higher risk for complicated pregnancies [68 69] and, as shown in this study, may also develop thyroid disease later in life.

As the study subjects in paper 4 had their miscarriages up to eighteen years earlier, we were also able to confirm their current health status as well as changes in health status across time. Women with thyroid disease at the time of the miscarriage, or even more evident, with current thyroid disease were clearly overrepresented among subjects with recurrent miscarriage in comparison with women who never miscarried. Although our controls may be younger when studied, giving a misleadingly low disease frequency in comparison with our cases, the prevalence of thyroid disorders in the general population is reported substantially lower (9 %) than found among our cases with recurrent miscarriages [70]. Our findings support the notion that repeated miscarriages may indicate subclinical thyroid disease, which ultimately may be diagnosed years after the miscarriages.

We have also, with relative certainty, confirmed that smokers have higher risk of repeated miscarriage [69]. However, it must be emphasized that the odds ratio for smoking may be overestimated as smoking gradually over the years has become more infrequent among Swedish women, resulting in lower smoking frequency in our controls. Whether smoking is a risk factor for spontaneous miscarriage is still a controversy [34, 71, 72] as self-reported data of a risk factor that is known to be harmful

during pregnancy may lead to reporting and recollection bias in either way, a factor that our data also may suffer from.

The small sample size of this study may be a limitation. On the other hand, to our knowledge, earlier studies have involved smaller number of cases, which makes our study unique. This investigation is furthermore strengthened by the large number of population based controls, residing in the same area as the cases. We were also able to match for age, an otherwise disturbing confounder.

The degree of progression of cervical dilatation during labour is the main indication of normal progression of labour. We have shown that when the cervical dilatation is normal, ERα mRNA expression in the lower uterine segment is significantly up-regulated compared to the mRNA levels after abnormal cervical dilatation, or in the uterus of women who are at term. (Paper 5) Although ERβ is highly expressed in the myometrium of women in term pregnancy, no correlation of the expression levels to cervical dilatation could be found. CYP7B1 did not differ between the three groups of women. The study also confirmed that the levels of 5AR1 were not correlated to progression of labour.

In the present study a local differentiated metabolism of estradiol could not be detected, since the cytochrome P450 19A1 (aromatase), which is the main enzyme that metabolizes androgens to estradiol, did not vary between the groups. The up-regulation of ERα seems to be the main way of regulation of estradiol effects on myometrium.

Earlier studies are inconsistent in their reporting of how ERα levels are affected during labour. This inconsistency may be explained by the fact that the progression of labour was not taken in to account [54, 73, 74].

A local metabolism leading to "progesterone withdrawal" in the uterus may be a possible mechanism leading to the onset of labour. The levels of AKR1C3 mRNA, the

main enzyme along with 5AR1 that metabolizes progesterone, were not altered in this study design, where the levels in the lower segment of the myometrium were measured.

Human labour is a complicated orchestration including the interplay between prostaglandins, oxytocin, progestins and estrogens. In this study, we could not find any correlation of androgen metabolising enzymes with progression of labour. Finally, the question remains, what mechanism/factor is actually conducting?

4 CONCLUSIONS

- Homozygous carriers of the UGT2B17 gene have significantly higher expression of the enzyme at the mRNA level in the prostate
- Lack of one allele of the UGT2B17 gene was associated with doubled risk of prostate cancer
- A novel polymorphism has been identified in the promoter region of the CYP7B1 gene, the gene was otherwise well-conserved.
- The allele frequency of this SNP on the CYP7B1 gene was too low, to substantially affect the prostate cancer risk among Swedes.
- The CYP1B1 Val432Leu polymorphism was associated with miscarriage in the first trimester although it is not a risk factor for repeated miscarriage.
- Repeated miscarriage is associated with future thyroid disease.
- Smoking, but not obesity is associated with repeated miscarriage
- Estrogen receptor alpha gene expression is associated with cervical ripening in the first stage of labour.
- No association with dysfunctional labour was found for the expression of ER beta, 5AR1, AKR1C1-4, CYP7B1, or aromatase.

5 ACKNOWLEDGEMENTS

Tiden är förbi då forskningsresultat kunde vara en enda kvinnas verk. Detta arbete är resultatet av flera personers ansträngningar som jag här vill tacka!

I första hand vill jag tacka alla kvinnor och män som deltagit i dem olika delarbetena. All forskning som handlar om människors sjukdomar kräver att flera försökspersoner generöst deltar. Denna avhandling hade aldrig kunnat bli av utan er insatts.

Jag vill tacka mina handledare:
Professor Anders Rane, min huvudhandledare som tog emot mig som ny forskare och har bidragit till min introduktion i forskningens värld.

Professor Britt-Marie Landgren, min bihandledare, som kom som en räddande ängel när jag mest behövde det. Du har bidragit till att även mitt intresse för gynekologi har kunnat bli tillgodosett och denna avhandling skulle aldrig blivit skriven utan din oerhört generösa hjälp och oöverträffbara kunskaper.

Mina medarbetare på Inst f Klin farmakolgi:
Docent Lena Ekström, vilkens kunskaper har varit i en ovärderlig hjälp för en ”kringirrande gynekolog i genetikens innersta värld”.

Laboratorievirtuosen Birgitta Ask, som turligt nog vägrat gå i pension, så att jag kunnat ha glädje av din långa erfarenhet på labbet.

Alla representanter för institutionen för kvinnors och barns hälsa vid Uppsala Universitet, särskilt:
Professor Inger Sundström Poromaa, som har varit till stor hjälp framförallt av delstudie 4 men också för din ”rockiga” inställning till forskning och för att du är en stor inspirationskälla för alla omkring dig.

Docent Anneli Stavreus-Evers som har varit min inofficiella handledare. Tusen tack för alla tips och handfast hjälp, denna avhandling skulle inte blivit av utan din hjälp.

Docent Helena Åkerud, vad skall man säga, inte trodde jag att så många pärlor kunde finnas vid samma institution! Du tillsammans med Eva Itzel-Wiberg har varit utmärkta och livsnödvändiga diskussionspartners i myometriestudien.

Professor Matts Olovsson chef för forskningslaboratoriet i Uppsala som inte uttalat någon protest alla dessa timmar som jag ockuperat labbet…

Professor Ove Axelsson, som en av initiativtagarna till den fantastiska biobanken för gravida i Uppsala, förutom Inger Sundström-Poromaa och Matts Olovsson. Jag vill även tacka dem forskningssköterskor som sköter biobanken, för utmärkt arbete.

Alla medarbetare i gruppen kring upprepade missfall, förutom Britt-Marie Landgren Inger Sundström Poromaa och Anneli Stavreus Evers även Katarina Bremme, Lottie Skjöldebrant-Sparre, Frida Husseini, Elisabeth Ljunger och Katja Lampinen. Tack för att vi har kunnat ha så givande, trevliga och roliga forskningsmöten.

Jag vill också särskilt tacka dem forskningssjuksköterskor/barnmorskor som hjälpt till med insamlandet av blodprover och noggrant bokfört allt som skall bokföras; Lena Moby i Uppsala, Margaretha Ström, Maria Karlsson och Maria Fursäter på Huddinge, Charlotte Wistrand Danderyd, Lotta Blomberg Solna.

Joel Kaplan, för fantastisk hjälp med språk granskning (inte av hela avhandlingen, får tilläggas då det inte hanns med, så ingen skugga över honom!).

Fredrik Tingstedt för engagerad och utmärkt it–support.

Docent Mia Wadelius för att ha bevarat mina myometriebiopsier på ett så utmärkt sätt, att denna avhandling kunde bli av och för att även jag kunnat ta del av Uppsala/Örebro studien.

Docent Eleni Aklillu och professor Leif Bertilsson, Huddinge för allt för kort forskningssammarbete, som för mig varit en givande inblick i forskningsvärlden.

Alla pigga, glada, snälla, kloka människor på forskningslabbet i Uppsala, som outtröttligt hjälpt mig när jag inte hittat ”av och på” knappen på Agilenten mm mm...

Alla människor på plan 8 på Huddinge för dito!

Alla mina övriga medförfattare: Anna Nordmark, Sven Cnattingius, Fredrik Granath, Mats Olsson, Jenny Jakobsson för givande forskningssammarbete.

Kollegorna på kvinnokliniken i Uppsala, nuvarande och tidigare, som hjälpt till med provinsamling av myometrieprover ”mitt i natten” och missfallspatienter!

Kollegerna på kvinnokliniken i Västerås, som gjorde den kliniska vardagen till en fest.

Verksamhetschef Bo Sultan för tjänstledighet inför denna avhandlings ”slutspurt”.
Min förra verksamhetschef Per Rymark på kvinnokliniken i Västerås, för förståelse av vad som krävs under ett avhandlingsarbete.

Heller Kieler för ”stjärtsparkar” och för att dragit ihop FDDF, hur skulle jag klarat mig utan er!

Min svärmor Laila Söderström som ställer upp för oss i ”vårt och torrt”.

Mina föräldrar Katarina och Charalampos Karypidis som lärt mig ”flytta berg”. Mina syskon Sappho, Nike och Grigoris som ”passat syskonbarn” och varit ”heja-klack”.

Mina älskade barn, Oscar och Andrée, som stått ut med ytterliggare en disputerande förälder… och kloka frågor som: ”Varför skall man disputera?”

Min älskade make Torbjörn, som fick mig att tro att detta skulle vara lätt. Du har varit min ständiga diskussionspartner och inspiratör!

Denna avhandling har i begynnelsen finansierats med forskningsanslag från Stiftelsen för strategisk forskning (SSF) via National network of drug development (NNDD).

6 REFERENCES

1. Geissler WM, Davis DL, Wu L, et al. Male pseudohermaphroditism caused by mutations of testicular 17 beta-hydroxysteroid dehydrogenase 3. Nat Genet 1994;7(1):34-9.

2. Mooradian AD, Morley JE, Korenman SG. Biological actions of androgens. Endocr Rev 1987;8(1):1-28.

3. Wylie K, Rees M, Hackett G, et al. Androgens, health and sexuality in women and men. Maturitas;67(3):275-89.

4. Arver S, Lehtihet M. Current guidelines for the diagnosis of testosterone deficiency. Front Horm Res 2009;37:5-20.

5. Diczfalusy E. The early history of estriol. J Steroid Biochem 1984;20(4B):945-53.

6. Jost A. Hormonal factors in the sex differentiation of the mammalian foetus. Philos Trans R Soc Lond B Biol Sci 1970;259(828):119-30.

7. Ferguson-Smith MA. Nuclear sex and the sex chromosomes. J Chronic Dis 1960;12:203-10.

8. Sinclair AH, Berta P, Palmer MS, et al. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature 1990;346(6281):240-4.

9. Josso N, Picard JY. Anti-Mullerian hormone. Physiol Rev 1986;66(4):1038-90.

10. Nordqvist K, Lovell-Badge R. Setbacks on the road to sexual fulfillment. Nat Genet 1994;7(1):7-9.

11. Siiteri PK, Wilson JD. Testosterone formation and metabolism during male sexual differentiation in the human embryo. J Clin Endocrinol Metab 1974;38(1):113-25.

12. Carlson AD, Obeid JS, Kanellopoulou N, Wilson RC, New MI. Congenital adrenal hyperplasia: update on prenatal diagnosis and treatment. The Journal of Steroid Biochemistry and Molecular Biology;69(1-6):19-29.

13. Ober C, Loisel DA, Gilad Y. Sex-specific genetic architecture of human disease. Nat Rev Genet 2008;9(12):911-22.

14. SchrÃ¶der FH, Hugosson J, Roobol MJ, et al. Screening and Prostate-Cancer Mortality in a Randomized European Study. New England Journal of Medicine 2009;360(13):1320-8.

15. Hugosson J, Carlsson S, Aus G, et al. Mortality results from the Göteborg randomised population-based prostate-cancer screening trial. The Lancet Oncology;11(8):725-32.

16. Stanford JL, Feng Z, Hamilton AS, et al. Urinary and Sexual Function After Radical Prostatectomy for Clinically Localized Prostate Cancer: The Prostate Cancer Outcomes Study. JAMA 2000;283(3):354-60.

17. Steineck G, Helgesen F, Adolfsson J, et al. Quality of Life after Radical Prostatectomy or Watchful Waiting. New England Journal of Medicine 2002;347(11):790-6.

18. Bray F, Klint A, Gislum M, et al. Trends in survival of patients diagnosed with male genital cancers in the Nordic countries 1964-2003 followed up until the end of 2006. Acta Oncol;49(5):644-54.

19. Stattin P, Holmberg E, Johansson JE, Holmberg L, Adolfsson J, Hugosson J. Outcomes in localized prostate cancer: National Prostate Cancer Register of Sweden follow-up study. J Natl Cancer Inst;102(13):950-8.

20. Bill-Axelson A, Holmberg L, FilÃ©n F, et al. Radical Prostatectomy Versus Watchful Waiting in Localized Prostate Cancer: the Scandinavian Prostate Cancer Group-4 Randomized Trial. Journal of the National Cancer Institute 2008;100(16):1144-54.

21. Huggins C, Hodges CV. Studies on prostatic cancer. I. The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. 1941. J Urol 2002;167(2 Pt 2):948-51; discussion 52.

22. Thompson IM, Goodman PJ, Tangen CM, et al. The Influence of Finasteride on the Development of Prostate Cancer. New England Journal of Medicine 2003;349(3):215-24.

23. Diczfalusy E. Endocrine Functions of the Human Fetoplacental Unit. Fed Proc 1964;23:791-8.

24. Pepe GJ, Albrecht ED. Actions of placental and fetal adrenal steroid hormones in primate pregnancy. Endocr Rev 1995;16(5):608-48.

25. Speroff LF, MA. Clinical Gynecologic Endocrinology and Infertility. 2005;Seventh edition:261-2.

26. Regan L, Braude PR, Trembath PL. Influence of past reproductive performance on risk of spontaneous abortion. Bmj 1989;299(6698):541-5.

27. Adolfsson A, Larsson PG. Cumulative incidence of previous spontaneous abortion in Sweden in 1983-2003: a register study. Acta Obstet Gynecol Scand 2006;85(6):741-7.

28. Stirrat GM. Recurrent miscarriage. Lancet 1990;336(8716):673-5.

29. Burgoyne PS, Holland K, Stephens R. Incidence of numerical chromosome anomalies in human pregnancy estimation from induced and spontaneous abortion data. Hum Reprod 1991;6(4):555-65.

30. Szabo I, Szilagyi A. Management of threatened abortion. Early Pregnancy 1996;2(4):233-40.

31. Ljunger E, Cnattingius S, Lundin C, Anneren G. Chromosomal anomalies in first-trimester miscarriages. Acta Obstet Gynecol Scand 2005;84(11):1103-7.

32. Clifford K, Rai R, Watson H, Regan L. An informative protocol for the investigation of recurrent miscarriage: preliminary experience of 500 consecutive cases. Hum Reprod 1994;9(7):1328-32.

33. Franssen MTM. Reproductive outcome after chromosome analysis in couples with two or more miscarriages: case-control study (vol 332, pg 759, 2006). British Medical Journal 2006;332(7548):1012-.

34. Maconochie N, Doyle P, Prior S, Simmons R. Risk factors for first trimester miscarriage--results from a UK-population-based case-control study. Bjog 2007;114(2):170-86.

35. Andersen A-MN, Wohlfahrt J, Christens P, Olsen Jr, Melbye M. Maternal age and fetal loss: population based register linkage study. BMJ 2000;320(7251):1708-12.

36. Ginsberg JS, Brill-Edwards P, Johnston M, et al. Relationship of antiphospholipid antibodies to pregnancy loss in patients with systemic lupus erythematosus: a cross-sectional study [see comments]. Blood 1992;80(4):975-80.

37. Empson M, Lassere M, Craig J, Scott J. Prevention of recurrent miscarriage for women with antiphospholipid antibody or lupus anticoagulant. Cochrane Database Syst Rev 2005(2):CD002859.

38. Lassere M, Empson M. Treatment of antiphospholipid syndrome in pregnancy--a systematic review of randomized therapeutic trials. Thromb Res 2004;114(5-6):419-26.

39. Kist WJ, Janssen NG, Kalk JJ, Hague WM, Dekker GA, de Vries JI. Thrombophilias and adverse pregnancy outcome - A confounded problem! Thromb Haemost 2008;99(1):77-85.

40. Visser J, Ulander VM, Helmerhorst FM, et al. Thromboprophylaxis for recurrent miscarriage in women with or without thrombophilia. HABENOX: A randomised multicentre trial. Thromb Haemost;105(2).

41. Kaandorp S, Di Nisio M, Goddijn M, Middeldorp S. Aspirin or anticoagulants for treating recurrent miscarriage in women without antiphospholipid syndrome. Cochrane Database Syst Rev 2009(1):CD004734.

42. Hemels MEH, Einarson A, Koren G, Lanctot KL, Einarson TR. Antidepressant Use During Pregnancy and the Rates of Spontaneous Abortions: A Meta-Analysis. Ann Pharmacother 2005;39(5):803-9.

43. Liddell HS, Pattison NS, Zanderigo A. Recurrent miscarriage--outcome after supportive care in early pregnancy. Aust N Z J Obstet Gynaecol 1991;31(4):320-2.

44. Devaseelan P, Fogarty PP, Regan L. Human chorionic gonadotrophin for threatened miscarriage. Cochrane Database Syst Rev (5):CD007422.

45. Haas DM, Ramsey PS. Progestogen for preventing miscarriage. Cochrane Database Syst Rev 2008(2):CD003511.

46. Johnson PM, Ramsden GH. Pregnancy immunology. Fetal and Maternal Medicine Review 1992;4(01):1-14.

47. Porter TF, LaCoursiere Y, Scott JR. Immunotherapy for recurrent miscarriage. Cochrane Database Syst Rev 2006(2):CD000112.

48. Liggins GC, Fairclough RJ, Grieves SA, Forster CS, Knox BS. Parturition in the sheep. Ciba Found Symp 1977(47):5-30.

49. Boroditsky RS, Reyes FI, Winter JS, Faiman C. Maternal serum estrogen and progesterone concentrations preceding normal labor. Obstet Gynecol 1978;51(6):686-91.

50. da Fonseca EB, Bittar RE, Carvalho MH, Zugaib M. Prophylactic administration of progesterone by vaginal suppository to reduce the incidence of spontaneous preterm birth in women at increased risk: a randomized placebo-controlled double-blind study. Am J Obstet Gynecol 2003;188(2):419-24.

51. Klebanoff MA, Meis PJ, Dombrowski MP, et al. Salivary progesterone and estriol among pregnant women treated with 17-alpha-hydroxyprogesterone caproate or placebo. Am J Obstet Gynecol 2008;199(5):506 e1-7.

52. Heine RP, McGregor JA, Goodwin TM, et al. Serial salivary estriol to detect an increased risk of preterm birth. Obstet Gynecol 2000;96(4):490-7.

53. Rezapour M, Backstrom T, Lindblom B, Ulmsten U. Sex steroid receptors and human parturition. Obstet Gynecol 1997;89(6):918-24.

54. Mesiano S, Chan EC, Fitter JT, Kwek K, Yeo G, Smith R. Progesterone withdrawal and estrogen activation in human parturition are coordinated by progesterone receptor A expression in the myometrium. J Clin Endocrinol Metab 2002;87(6):2924-30.

55. Quinn MA, Murphy AJ, Kuhn RJ, Robinson HP, Brown JB. A double blind trial of extra-amniotic oestriol and prostaglandin F2 alpha gels in cervical ripening. Br J Obstet Gynaecol 1981;88(6):644-9.

56. Thiery M, De Gezelle H, Van Kets H, et al. Extra-amniotic oestrogens for the unfavourable cervix. Lancet 1978;2(8094):835-6.

57. Magann EF, Perry KG, Dockery JR, Bass JD, Chauhan SP, Morrison JC. Cervical ripening before medical induction of labor: a comparison of prostaglandin E2,

estradiol, and oxytocin. American Journal of Obstetrics and Gynecology 1995;172(6):1702-8.

58. Stewart P, Kennedy JH, Barlow DH, Calder AA. A comparison of oestradiol and prostaglandin E2 for ripening the cervix. Br J Obstet Gynaecol 1981;88(3):236-9.

59. Gifford DS, Morton SC, Fiske M, Keesey J, Keeler E, Kahn KL. Lack of progress in labor as a reason for cesarean. Obstet Gynecol 2000;95(4):589-95.

60. Thornton JG, Lilford RJ. Active management of labour: current knowledge and research issues. Bmj 1994;309(6951):366-9.

61. Wei S, Wo BL, Xu H, Luo ZC, Roy C, Fraser WD. Early amniotomy and early oxytocin for prevention of, or therapy for, delay in first stage spontaneous labour compared with routine care. Cochrane Database Syst Rev 2009(2):CD006794.

62. Livak KJ. Allelic discrimination using fluorogenic probes and the 5' nuclease assay. Genet Anal 1999;14(5-6):143-9.

63. Park J, Chen L, Ratnashinge L, et al. Deletion polymorphism of UDP-glucuronosyltransferase 2B17 and risk of prostate cancer in African American and Caucasian men. Cancer Epidemiol Biomarkers Prev 2006;15(8):1473-8.

64. Olsson M, Lindstrom S, Haggkvist B, et al. The UGT2B17 gene deletion is not associated with prostate cancer risk. Prostate 2008;68(5):571-5.

65. Gallagher CJ, Kadlubar FF, Muscat JE, Ambrosone CB, Lang NP, Lazarus P. The UGT2B17 gene deletion polymorphism and risk of prostate cancer. A case-control study in Caucasians. Cancer Detect Prev 2007;31(4):310-5.

66. Kesarwani P, Mittal RD. Selection of inappropriate controls in lieu of paper published by Gallagher et al. [Cancer Detect Prev 2007;31 (4): 310-315]. Cancer Detection and Prevention 2008;32(3):185-.

67. Saijo Y, Sata F, Yamada H, et al. Ah receptor, CYP1A1, CYP1A2 and CYP1B1 gene polymorphisms are not involved in the risk of recurrent pregnancy loss. Mol Hum Reprod 2004;10(10):729-33.

68. van Oppenraaij RHF, Jauniaux E, Christiansen OB, Horcajadas JA, Farquharson RG, Exalto N. Predicting adverse obstetric outcome after early pregnancy events and complications: a review. Human Reproduction Update 2009;15(4):409-21.

69. Bhattacharya S, Townend J, Bhattacharya S. Recurrent miscarriage: Are three miscarriages one too many? Analysis of a Scottish population-based database of 151,021 pregnancies. Eur J Obstet Gynecol Reprod Biol;150(1):24-7.

70. Bjoro T, Holmen J, Kruger O, et al. Prevalence of thyroid disease, thyroid dysfunction and thyroid peroxidase antibodies in a large, unselected population. The Health Study of Nord-Trondelag (HUNT). Eur J Endocrinol 2000;143(5):639-47.

71. Chatenoud L, Parazzini F, di Cintio E, et al. Paternal and maternal smoking habits before conception and during the first trimester: relation to spontaneous abortion. Ann Epidemiol 1998;8(8):520-6.

72. van Ravenswaaij R, Tesselaar-van der Goot M, de Wolf S, van Leeuwen-Spruijt M, Visser GH, Schielen PC. First-trimester serum PAPP-A and fbeta-hCG concentrations and other maternal characteristics to establish logistic regression-based predictive rules for adverse pregnancy outcome. Prenat Diagn.

73. Wu JJ, Geimonen E, Andersen J. Increased expression of estrogen receptor beta in human uterine smooth muscle at term. Eur J Endocrinol 2000;142(1):92-9.

74. Winkler M, Kemp B, Classen-Linke I, et al. Estrogen receptor alpha and progesterone receptor A and B concentration and localization in the lower uterine segment in term parturition. J Soc Gynecol Investig 2002;9(4):226-32.