Anti-Müllerian Hormone Levels in Patients with Unexplained Recurrent Spontaneous Abortions

A thesis
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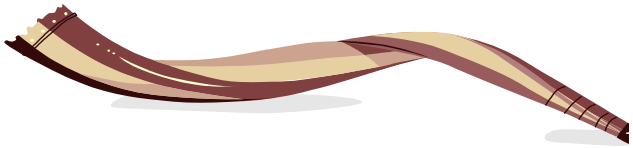

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LIST OF ABBREVIATIONS

aCL	anticardiolipin antibodies
AFC	Antral follicle count
ALK	Anaplastic lymphoma kinase
AMH	Anti-Müllerian hormone.
AMHRII	AMH type II receptor
Anti-/32g1	Anti-/32 glycoprotein 1
Anti-beta-2GPI	Anti-beta2 glycoprotein I
APAS	Antiphospholipid antibody syndrome
ARDS	Adult respiratory distress syndrome
ASRM	American Society for Reproductive Medicine
AUC	Area under curve
BV	Bacterial vaginosis
CCCT	Clomiphene citrate challenge test
CMV	Cytomegalovirus
D&C	Dilatation and curretage.
DES	Di-ethyl stillbisterol
E_2	Estradiol
EGF	Epithelial growth factor
FSH	Follicle stimulating hormone
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
hMG	human menopausal gonadotropin
HSG	hysterosalpingogram .
IGF2	insulin-like growth factor 2
Insl3	Insulin-like factor 3
IQR	Inter-quartile range
IUFD	Intrauterine fetal demise
IUGR	Intrauterine growth restriction
IVIg	Intravenous immunoglobulins
LAs	lupus anticoagulants
LH	leuteinizing hormone
LMWH	low-molecular-weight heparin

LIST OF ABBREVIATIONS (Cont.)

LPD	Luteal phase deficiency
MIS	Müllerian inhibiting substance
MRI	Magnetic resonance imaging
MTHFR	Methylene tetrahydrofolate reductase
NK	Natural killer
ORTs	Ovarian reserve tests
PCOS	Polycystic ovarian syndrome
PIH	Pregnancy-induced hypertension
RCOG	Royal College of Obstetricians
RPL	Recurrent pregnancy loss
SLE	systemic lupus erythematosus
TGFß	Transforming growth factor-B
TNFα	Tumor necrosis factor alpha
VEGF	vascular endothelial growth factor
VTE	Venous Thromboembolism

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INTRODUCTION

Abortion is one of the most common complications of pregnancy, occurring in 10–15% of pregnant women. It is defined as pregnancy that fails to progress resulting in death of the fetus before age of fetal viability (20^{th} week (weight of ≤ 500 g) in developed countries and 28^{th} week (weight of ≤ 1 kg) in developing countries).

Recurrent spontaneous abortion, rate of 2–5%, is defined as ≥ 3 spontaneous abortions. In these women, it is necessary to conduct a comprehensive evaluation so that a plan of care can be outlined **(Daya, 2004).**

Recently, the Practice Committee of the American Society for Reproductive Medicine (ASRM) in January 2008 has defined recurrent pregnancy loss by two or more failed pregnancies. When the cause is unknown, each pregnancy loss merits careful review to determine whether specific evaluation may be appropriate. After three or more losses, a thorough evaluation is warranted. For purposes of determining when evaluation and treatment for infertility or for recurrent pregnancy loss is appropriate, pregnancy is defined as a clinical pregnancy documented by ultrasonography or histopathologic examination **(ASRM, 2008).**

Antimüllerian hormone (AMH) is a dimeric glycoprotein made up of two monomers attached to each other by disulfide bonds. The 72-kd molecule belongs to the transforming growth factor-B superfamily, which acts on tissue growth and differentiation. Sertoli cells in the male produce AMH, which induces the degeneration of the müllerian ducts and provides the normal formation of the male genital system. Sertoli cells' secretion of AMH continues for a lifetime, but the significance of AMH in adult male is not known. In females, the granulosa cells of the ovary express AMH postnatally. Serum AMH levels are lower in females

compared with males. After puberty and onset of menstrual cycles, serum AMH level decreases progressively until it becomes undetectable at around menopause **(Gruijters et al., 2003).**

Anti-Müllerian hormone can first be detected in human fetal ovary at 36th week in columnar granulosa cells of maturing primary follicles**.** AMH expression persists in these follicles and is maximally expressed in granulosa cells of preantral and small antral follicles (up to 6 mm). At larger antral follicle stage (>8 mm), its expression diminishes and ultimately becomes undetectable once FSH dependent follicular growth has been initiated. No AMH expression is detected in atretic follicles **(Broekmans et al., 2006).**

Secreted from preantral and early antral follicles, AMH regulates ovarian activity and follicular steroidogenesis. Animal studies have revealed that not only does AMH decrease aromatase activity of FSH-stimulated granulosa cells, but it also decreases the number of leuteinizing hormone (LH) receptors, and regulates testosterone production in theca cells **(Cook et al., 2000).**

The regulation of oocyte function is through endocrine and paracrine factors. Although the basic hormones causing oocyte growth are FSH and LH, receptors for gonadotropins could not be found on oocytes. The action of gonadotropins on oocytes is probably through mediators like epithelial growth factor (EGF), vascular endothelial growth factor (VEGF), insulin-like growth factor 2 (IGF2). The intrafollicular androgen to estrogen ratio also acts on oocyte function, and AMH plays a major role in the regulation of this ratio **(Fiçiciog et al., 2000).**

The pattern of expression of AMH strongly indicates that AMH has an important role in regulating the number of follicles that grow from the primordial pool. Moreover, AMH

might regulate selection of the dominant follicle from the FSH-sensitive follicle cohort **(Broekmans et al., 2008).**

Serum AMH levels were more robustly correlated with the number of early antral follicles than E_2, FSH and LH on cycle day 3. This suggests that AMH may reflect ovarian follicular status better than the usual hormone markers **(Fanchin et al., 2007).**

On the other hand, ovarian reserve is a term used to describe the functional potential of the ovary and reflects the number and quality of oocytes within it **(Macklon and Fauser, 2005)**.

AMH was the best indicator of ovarian reserve with a high sensitivity and specificity. Levels of AMH would predict the number of oocytes with a positive predictive rate of 96%, although it had little value for predicting pregnancy **(Fiçiciog et al., 2000).**

The process of follicular recruitment and selection takes place during the follicular phase of the cycle. Hence, events during the follicular phase may affect the pregnancy outcome. However, most studies on recurrent abortion have focused on the luteal phase of menstrual cycle, and there is limited information on the follicular phase and correlated ovarian reserve in women with recurrent abortions, especially cases with unexplained causes **(Parakash et al., 2006).**

The aim of this work is to:

Evaluate whether follicular phase ovarian reserve of patients with unexplained recurrent spontaneous abortions is defective or not by measuring serum levels of anti-Müllerian hormone during ovarian follicular phase.

CHAPTER ONE
Recurrent spontaneous abortion

Abortion is one of the most common complications of pregnancy, occurring in 10–15% of pregnant women. It is defined as pregnancy that fails to progress resulting in death of the fetus before age of fetal viability 20^{th} week (weight of fetus ≤ 500 g) in developed countries and 28^{th} week (weight of fetus ≤ 1 kg) in developing countries). Recurrent spontaneous abortion, rate of 2–5%, is defined as ≥ 3 spontaneous abortions (consecutive or not). In these women, it is necessary to conduct a comprehensive evaluation so that a plan of care can be outlined **(Daya, 2004).**

Subgroups of recurrent miscarriage:

Based on the pregnancy history, three different groups of women with recurrent miscarriage can be identified, and the risk of subsequent miscarriage among these groups varies **(Daya, 2000):**

(i) **Primary recurrent miscarriage group**. This group consists of women with three or more consecutive miscarriages with no pregnancy progressing beyond 20 weeks' gestation.

(ii) **Secondary recurrent miscarriage group.** This group consists of women who have had three or more consecutive miscarriages following at least one pregnancy that has gone beyond 20 weeks' gestation, and may have ended in live birth, stillbirth, or neonatal death.

(iii) **Tertiary recurrent miscarriage group.** This group has not been well studied and consists of women who have had at least three miscarriages that are not consecutive but are interspersed with pregnancies that have progressed beyond 20 weeks' gestation (and may have ended in live birth, stillbirth, or neonatal death).

The current approach of lumping all three groups together makes it difficult to make recommendations regarding optimal evaluation and management.

Risk Factors

Age and success of previous pregnancies are two independent risk factors that affect the loss rate. Many authors have observed an increasing risk of fetal death, in particular spontaneous abortion, with increasing maternal age **(Salim et al., 2003).** The association of age of the mother and the increased likelihood of chromosomal abnormalities is manifested by the age related increase of trisomy 21 and cytogenetic studies on preimplantation embryos **(Salim et al., 2003).** Outcome of previous pregnancies is another decisive factor in the risk of pregnancy loss. For young women who have never experienced a loss, the rate of a clinical miscarriage is as low as 5% **(Patel et al., 1997).**

The risk increases to approximately 30% for women with three or more losses but with a previous live-born infant **(Shortle and Jewelewicz, 1989)** and up to 50% for women without a live-born infant **(Schenker and Margolith, 1982).** From these data, it is evident that some women are at particular risk for losing their pregnancy and that there must be an underlying cause for it. Before dealing with possible mechanisms of recurrent miscarriage, it should be remembered that investigations are necessarily confounded by the fact that the same mechanisms as those in sporadic miscarriage can be involved. The same uncertainty applies for the evaluation of any treatment. It is estimated that approximately 33% of women with so called recurrent miscarriage will have had three consecutive sporadic miscarriages by chance **(Li et al., 2000).**