Serum Antimullerian Hormone and Ovarian Follicular Blood Flow as Predictors of Low Response in Intracytoplasmic Sperm Injection

Thesis

Submitted for partial fulfilment of the
MD degree in Obstetrics & Gynaecology

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2009

Acknowledgements

I would like to express my thanks to ***Prof. Essam El-Din Mohammed Ammar****, professor of obstetrics & gynaecology, for his support and guidance during the course of this work.*

I would also like to express my deep gratitude to ***Prof. Mohamed Ashraf Mohamed Farouk Kortam****, professor of obstetrics & gynaecology, for his immense effort in the scientific and written work of this thesis, and whose patience and perseverance was the cornerstone in producing this work.*

I would also like to thank ***Prof. Mona Fathy Youssef****, professor of clinical pathology, for her extremely valuable work in the laboratory aspect of the thesis, without which this work would never have been accomplished.*

Deep thanks are also due to ***Prof. Hesham Mahmoud Harb****, professor of obstetrics and gynaecology, for his support during the work.*

And last, but certainly not least, I would like to express my deep thanks to the staff of the ***Unit of Assisted Reproduction****, Ain Shams University Maternity Hospital, for their help in the practical aspect of this study.*

Table of Contents

List of Tables

Tables in the Review of Literature

Tables in the Results

List of Figures

Figures in the Review of Literature

Figures in the Results

List of Abbreviations

Abbreviation	*Term*
ALK	Activin receptor-like kinase
AMH	Anti-Mullerian hormone
AMHKO	Anti-Mullerian hormone knockout
AMHR	Anti-Mullerian hormone receptor
ART	Assisted reproductive technology
ASRM	American Society for Reproductive Medicine
BBT	Basal body temperature
bFGF	Basic fibroblast growth factor
BMP	Bone morphogenetic protein
bp	Base pairs
cAMP	Cyclic adenosine monophosphate
CHO	Chinese hamster ovary
CI	Confidence interval
COX	Cyclooxygenase
Cx	Connexin
Dax-1	Dosage-sensitive sex reversal critical region on the X chromosome gene-1
DNA	Deoxyribonucleic acid
E_2	Oestradiol
EGF	Endothelial growth factor
ELISA	Enzyme-linked immunosorbent assay
FSH	Follicle stimulating hormone
GDF	Growth differentiation factor
GDP	Guanosine diphosphate
GIFT	Gamete intrafallopian transfer
GTP	Guanosine triphosphate
GnRH	Gonadotrophin releasing hormone
hCG	Human chorionic gonadotrophin
HDL	High density lipoprotein
hMG	Human menopausal gonadotrophins

HRP	Horseradish peroxidase
HSD	Hydroxysteroid dehydrogenase
HSG	Hysterosalpingography
ICSI	Intracytoplasmic sperm injection
IGF	Insulin-like growth factor
IUI	Intrauterine insemination
IVF	In vitro fertilization
kDa	Kilo Daltons
LDL	Low density lipoprotein
LH	Luteinizing hormone
MESA	Microsurgical epididymal sperm aspiration
MIS	Mullerian inhibitory substance
Nor	Nuclear corepressor
NGF	Nerve growth factor
OR	Odds ratio
PCOD	Polycystic ovarian disease
PGD	Pre-implantation genetic diagnosis
PKC	Protein kinase C
PMDS	Persistent Mullerian duct syndrome
PR	Progesterone receptor
PTK	Protein tyrosine kinase
PVP	Polyvinylpyrrolidone
RNA	Ribonucleic acid
ROC	Receiver operating characteristic
rpm	Rounds per minute
SCF	Stem cell factor
SART	Society for Assisted Reproductive Technology
SF	Steroidogenic factor
SRY	Sex determining region of the Y chromosome
StAR	Steroid acute regulatory protein
T	Testosterone
TESE	Testicular sperm extraction
TGFβ	Transforming growth factor beta
TIC	Theca interstitial cells
TMB	Tetramethylbenzidine
WHO	World Health Organisation
WT1	Wilm’s tumour suppressor

ZIFT	Zygote intrafallopian transfer
ZP	Zona pellucida protein

Abstract

Studies on the use of Antimullerian hormone (AMH) as a marker for ovarian ageing in women have recently started. As AMH is largely expressed throughout folliculogenesis, from the primary follicular stage towards the antral stage, serum levels of AMH may represent both the quantity and quality of the ovarian follicle pool. The development of vaginal colour and pulse Doppler ultrasound has resulted in the recent tendency to evaluate the quality and the developmental competence of the oocyte using the ovarian vascular change in women undergoing IVF. It has been reported that perifollicular blood flow characteristics could be a marker for evaluating the quality of oocytes and the developmental competence of the corresponding embryos. A study was conducted in the Assisted Reproduction Unit, Ain Shams Maternity Hospital. It included 50 infertile women undergoing intracytoplasmic sperm injection (ICSI) for treatment of infertility. All women were subjected to pituitary downregulation using either the short or long protocols applied by the unit. On day 3 of the induction cycle, serum AMH was measured and a transvaginal ultrasound scan was performed to determine the number of antral follicles and the mean ovarian volume. On the day of HCG administration, serum AMH was measured again and perifollicular blood flow was assessed using colour Doppler transvaginal ultrasound. Results showed that the number of good responders was 37 (74%) and poor responders were 13 (26%). Both day-3 and end-of-cycle AMH levels were significantly higher ($p<0.01$) in the good response group. However, the difference between day-3 AMH and end-of-cycle AMH was non-significant ($p>0.05$). There was a highly significant ($p<0.01$) difference between the two groups as regards Doppler resistance index, being higher in the poor response group. There was also a highly significant positive correlation ($p>0.05$) between serum AMH and both the number of retrieved oocytes and the number of grade 1 embryos. There was a highly significant negative correlation between Doppler resistance index and both the number of retrieved oocytes and the number of grade 1 embryos. There was a highly significant positive correlation ($p<0.01$) between serum AMH and both antral follicle count and mean ovarian volume. The negative correlations between Doppler resistance index and both antral follicle count and mean ovarian volume proved to be statistically significant ($p<0.05$) and highly significant ($p<0.01$), respectfully. The negative correlation between serum AMH and Doppler resistance index was highly significant ($p<0.01$). Serum AMH showed the highest sensitivity (97.3%) and specificity (92.3%) as a test for prediction of ovarian response. On the other hand, the Doppler resistance index, despite showing high sensitivity (97.3%), showed a very low specificity (7%).

Introduction

The concept of ovarian reserve is loosely defined as the size and quality of the remaining ovarian follicular pool. The number of oocytes in any given woman is genetically determined and inexorably declines throughout life, from approximately 1-2 million at birth to about 300,000 at puberty, 25,000 at age 37-38 (when the pace of follicular depletion accelerates), and fewer than 1000 at menopause **(Faddy *et al,* 1992 and Gougeon *et al*, 1994)**.

Poor response in IVF cycles is a cause of cycle cancellation and to repeated attempts for a better response in subsequent cycles **(Vladimirov *et al.*, 2005)**. Treatment cancellation because of poor ovarian response is a significant problem. The prevalence of poor response is estimated to be 9-24% of the ART population **(Tarlatzis *et al.*, 2003)**.

A poor ovarian response is a reflection of reduced total ovarian follicular capacity (reserve). Identification of women with a markedly reduced ovarian reserve, likely to lead to cycle cancellation, is paramount prior to embarking on expensive and invasive treatment such as IVF **(Akande *et al.*, 2004)**. Ovarian reserve tests help to predict the response to exogenous gonadotrophin stimulation and the likelihood of success with IVF and are widely accepted as an essential element of the evaluation of IVF candidates. Considering the associated costs, logistics, and risks, accurate prognostic information is very helpful to couples who may be considering IVF. Ovarian reserve tests are generally reliable, but not infallible. A number of methods for measuring ovarian reserve have been described **(Speroff and Fritz, 2005)**.

Laboratory tests of ovarian reserve include basal serum FSH and oestradiol levels, in addition to inhibin-B levels **(Vladimirov *et al.*, 2005 and McIlveen *et al.*, 2007)**.

Studies on the use of Antimullerian hormone (AMH) as a marker for ovarian ageing in women have recently started. As AMH is largely expressed throughout folliculogenesis, from the primary follicular stage towards the antral stage, serum levels of AMH may represent both the quantity and quality of the ovarian follicle pool **(La Marca and Volpe, 2006)**. Studying young normo-ovulatory women showed that serum concentrations of AMH change over time and with advancing age before changes occur in other currently known ageing-related variables, such as serum concentrations of FSH, inhibin B and oestradiol **(de Vet *et al.*, 2002, van Rooij *et al.*, 2002, Hazout *et al.*, 2004, Muttukrishna *et al.*, 2004, Tremellen *et***

***al*., 2005, van Rooij *et al*., 2005, Visser *et al*., 2006 and Nakhuda *et al*., 2007)**.

Furthermore, the relationship between serum concentrations of AMH and the number of antral follicles **(van Rooij *et al*., 2002, Muttukrishna *et al*., 2005, Lekamge *et al*., 2007 and McIlveen *et al*., 2007)** and mean ovarian volume **(McIlveen *et al*., 2007)** has been studied in women undergoing IVF treatment.

Ultrasound is also a helpful tool in the prediction of poor response. The measurement of ovarian volume **(Fratarelli *et al*., 2004, Hendriks *et al*., 2007 and Kwee *et al*., 2007)** and antral follicle count **(Klinkert *et al*., 2005, Hendriks *et al*., 2007, Kwee *et al*., 2007 and Soldevila *et al*., 2007)** have been studied as predictors of ovarian reserve.

The development of vaginal colour and pulse Doppler ultrasound has resulted in the recent tendency to evaluate the quality and the developmental competence of the oocyte using the ovarian vascular change in women undergoing IVF **(Engmann *et al*., 1999)**. The practical value of these studies is still unproven. Increase in ovarian blood flow is related to angiogenesis, which starts early in the menstrual cycle when the dominant follicle or the dominant ovary has been selected. The gradual increase in ovarian blood flow is related to increasing oestrogen levels, and the dramatic increase that is noted to be preovulatory is most likely to be related to a sudden increase in intrafollicularRIY progesterone levels **(Goswamy, 2005)**.

It has been reported that perifollicular blood flow characteristics could be a marker for evaluating the quality of oocytes and the developmental competence of the corresponding embryos **(Coulam *et al.,* 1999, Battaglia *et al.,* 2000 and Kim *et al.,* 2004)**.