

Follicular Output RaTe (FORT) may predict Intra Cytoplasmic Sperm Injection (ICSI) outcome

Thesis

**Submitted for partial fulfillment of Master Degree in
Obstetrics &Gynecology**

By

Omar Gamal Farag Mustafa

M.B.B.CH., Faculty of Medicine, Ain Shams University, 2014
Obs.& Gyn.Registrar at Dar EL Shefa Hospital
(Ministry of Health Specialized Medical Centers)

Under Supervision of

Prof. Hesham Mohamed Fathy

Professor of Obstetrics and Gynaecology
Faculty of Medicine - Ain Shams University

Prof. Sherif Fathi El-Mekkawi

Professor of Obstetrics and Gynaecology
Faculty of Medicine - Ain Shams University

Dr. Ahmed Mohamed El-kotb

Lecturer of Obstetrics and Gynaecology
Faculty of Medicine - Ain Shams University

**Faculty of Medicine
Ain Shams University
2018**

Acknowledgement

*First, and foremost, my deepest gratitude and thanks should be offered to **ALLAH**, the Most Kind and Most Merciful, for giving me the strength to complete this work.*

*I would like to express my sincere gratitude to **Prof. Dr. Hesham Mohamed Fathy**, Professor of Obstetrics and Gynaecology, Faculty of Medicine, Ain Shams University for his continuous support and guidance for me to present this work. It really has been an honor to work under his generous supervision.*

*I would like to express my deepest appreciation to **Prof. Dr. Sherif Fathi El-Mekkawi** Professor of Obstetrics and Gynaecology, Faculty of Medicine, Ain Shams University for his endless patient, enormous effort and faithful supervision through out the work.*

*I acknowledge with much gratitude to **Dr. Ahmed Mohamed El-kotb**, Lecturer of Obstetrics and Gynaecology, Faculty of Medicine, Ain Shams University, for his great supervision, efforts and unlimited help to provide all facilities in the whole work.*

*Last but not least, thanks to all members of **Assisted Reproduction Unit** – Ain Shams University Maternity Hospital and other sharing centers for helping me to finish this work.*

List of Contents

	<i>Page</i>
List of Abbreviations	i
List of Tables.....	ii
List of Figures	iv
Protocol	
Introduction	1
Aim of the Work	3
Review of Literature.....	4
Chapter (1): Follicle Growth and Development ...	4
Chapter (2): Ovarian Reserve	35
Chapter (3): Ovarian Reserve Assessment.....	47
Chapter (4): Controlled Ovarian Stimulation in IVF/ICSI.....	74
Chapter (5): Prediction of Ovarian Response	79
Chapter (6): In Vitro Fertilization /ICSI (Prediction of Outcome)	84
Patients and Methods	93
Results	104
Discussion	126
Summary	134
Conclusions	137
References	139
Arabic Summary	

List of Abbreviations

2D	Two dimension
3D	Three Dimension
AECC	Autologous Endometrial Co-Culture
AFC	Antral Follicle Count
AMH	Anti Mullerian Hormone
ANOVA	Analysis Of Variance
ARC	Assisted Reproduction Clinics
ART	Assisted Reproduction Techniques
ASRM	American Society for Reproductive Medicine
B-FSH	Basal Follicle Stimulating Hormone
BMI	Body Mass Index
C AMP	Cyclic Adenosine MonoPhosphate
CAH	Congenital Adrenal Hyperplasia
CC	Clomiphene Citrate
CCCT	Clomiphene Citrate Challenge Test
CD	Color Doppler
CDE	Color Doppler Energy
CI	Confidence Interval
COH	Controlled Ovarian Hyperstimulation
COS	Controlled Ovarian Stimulation
Cx37	Connexin 37
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone sulfate
DNA	DeoxyriboNucleic Acid
EFORT	Exogenous FSH Ovarian Reserve Test
ESHRE	European Society of Human Reproduction and Embryology
ET	Embryo Transfer
FAI	Free androgen index
FMR 1	Fragile X Mental Retardation
FORT	Follicular Output RaTe
FSH	Follicle Stimulating Hormone
GAST	GnRH Agonist Stimulation Test
GDF9	Growth Differentiation Factor 9
GIFT	Gamete IntraFallopian Transfer
GnRH	Gonadotrophin Releasing Hormone
GNRHa	Gonadotrophin Releasing Hormone Analogue
GNSAF	gonadotrophin surge attenuating factor
GVBD	Germinal vesicle BreakDown

HCG	Human Chorionic Gonadotropin
HMG	Human Menopausal Gonadotrophin
HSG	HysteroSalpingoGram
ICSI	Intracytoplasmic sperm injection
IGF-1	Insulin like Growth Factor 1
IU	International Unit
IVF	InVitro Fertilization
KDa	Kilo Dalton
LH	Lutenizing Hormone
MII	Metaphase two
MOV	Mean Ovarian Volume
Mrna	Mitochondrial RiboNucleic Acid
NRO	Number of Oocytes
OH	Office Hysteroscope
OHSS	Ovarian HyperStimulation Syndrome
OPU	Oocyte Pick Up
OR	Ovarian Reserve
OR	Odds Ratio
ORT	Ovarian Reserve Test
PFC	Pre ovulatory Follicule Count
PI	Pulsatile Index
POF	Premature Ovarian Failure
POI	Premature Ovarian Insufficiency
POR	Poor Ovarian Response
QE2	Estradiol
RNA	RiboNucleic Acid
SD	Standard Deviation
SHBG	Sex Hormone Binding Globulin
STROBE	STrengthening the Reporting of Of Observational studies in Epidemiology
TGF B	Transfoming Growth Factor Beta
TICs	Theca Interstitial cells
TV U/S	Trans Vaginal Ultra Sound
VEGF	Vascular Endothelial Growth Factor
VIP	Vital Initiation of Pregnancy
VOCAL	Virtual Organ Computer Aided Analysis
WHO	World Health Organization
ZP	Zona Pellucida

List of Tables

Table	Title	Page
1	Cytotoxic agents according to the degree of gonadotoxicity.	42
2	The descriptive values for antral follicle count that are stratified according to the female age.	65
3	The percentage of antral follicle count according to each age.	66
4	How many antral follicles are good?	67
5	Advantages of specific protocols and clinical scenarios in which they may be preferred.	76
6	Comparison between study groups regarding demographic and clinical characteristics.	109
7	Comparison between study groups regards basic IVF/ICSI cycle characteristics.	111
8	Comparison between study groups regards follicular recruitment dynamics during IVF/ICSI cycle.	114
9	Comparison between study groups regards oocyte/embryo outcomes from IVF/ICSI cycle.	116
10	Comparison between tubal-factor women who achieved clinical pregnancy and those who didn't; regarding follicular recruitment parameters and oocyte/embryo outcomes.	120
11	Comparison between endometriosis women who achieved clinical pregnancy and those who didn't; regarding follicular recruitment parameters and oocyte/embryo outcomes.	122
12	Comparison between unexplained infertility women who achieved clinical pregnancy and those who didn't; regarding follicular recruitment parameters and oocyte/embryo outcomes.	124

List of Figures

Fig.	Title	Page
1	Photomicrograph of ovulation shows the expanded egg-cumulus complex leaving the follicle through the stigma.	5
2	Chronology of folliculogenesis in human ovaries.	8
3	Photomicrograph of an adult primate ovary.	9
4	Electron micrograph of a human primordial follicle shows the flattened granulosa cells (GC), the oocyte with its germinal vesicle (GV) or nucleus, the Balbiani body (BB), with all the oocyte organelles gathered at one pole of the GV, and basal lamina (BL) .	9
5	Age-dependent changes in the number of primordial follicles (oocytes) in human ovaries	10
6	The age-related decrease in the number of primordial follicles (PF) within both human ovaries from birth to the menopause.	11
7	Photomicrograph of nongrowing primordial and a newly recruited (growing) follicle in the human ovary. Notice the cuboidal granulosa cells (arrowheads) in the newly recruited primordial follicle.	12
8	Relation between granulosa number in the largest cross section of the follicle and the distribution of flattened and cuboidal cells.	13
9	Diagram illustrating the size and histologic organization of early developing human follicles during the gonadotropin-independent period of folliculogenesis.	14
10	Drawing of a developing primary follicle embedded in the connective tissue or stroma of the ovary cortex.	15

Fig.	Title	Page
11	Diagram of the proposed mechanism for the autocrine control of follicle-stimulating hormone receptor expression in granulosa cells of preantral follicles.	16
12	Electron micrograph of the oocyte-corona radiata granulosa cells in a preantral follicle.	18
13	A typical healthy secondary follicle contains a fully grown oocyte surrounded by the zona pellucida, five to eight layers of granulosa cells, a basal lamina, and developing theca tissue with numerous blood vessels.	19
14	Photomicrograph of an early tertiary follicle (0.4 mm in diameter) at the cavitation of early antrum stage.	21
15	Photomicrograph of a polyovular follicle at the early tertiary stage shows the sites of cavitation or early antrum formation (clear spaces) just above oocytes (asterisk).	22
16	Diagram of the architecture of a typical class 5 Graafian follicle.	23
17	Drawing of the wall of a Graafian follicle.	24
18	Diagram of the structure and function heterogeneity of the granulosa cells in a healthy Graafian follicle.	25
19	The two major classes of Graafian follicles: healthy and atretic.	27
20	Diagram of the life cycle of Graafian follicles in human ovaries	28
21	Changes in the number of granulosa cells and volume of follicular fluid in human graafian follicles throughout the course of folliculogenesis.	30
22	The luteal-follicular transition in women.	32

Fig.	Title	Page
23	Illustration of the concept that the dominant follicle contains relatively high levels of follicle-stimulating hormone (FSH) in the follicular fluid, whereas FSH levels are low or undetectable in cohort follicles destined for atresia.	33
24	Decline rate in the OV (ml per year) and AF count (n per year) with age.	39
25	Variations in age at the occurrence of specific stages of ovarian ageing.	40
26	Polycystic ovary (B mode transvaginal route).	43
27	COS protocols.	76
28	Flow diagram showing the recruitment and handling of the study population during the course of the study.	106
29	Bar graph summarizing demographic and clinical data of the study groups.	108
30	Bar graph summarizing basal hormonal profile and AFC in the four study groups.	112
31	Bar graph summarizing follicular recruitment dynamics in the four study groups.	115
32	Bar graph summarizing oocyte/embryo outcomes in the four study groups.	117
33	Bar graph summarizing follicular recruitment parameters and oocyte/embryo outcomes in PCOS women who achieved clinical pregnancy and those who didn't.	119
34	Bar graph summarizing follicular recruitment parameters and oocyte/embryo outcomes in tubal-factor women who achieved clinical pregnancy and those who didn't.	121

Fig.	Title	Page
35	Bar graph summarizing follicular recruitment parameters and oocyte/embryo outcomes in endometriosis women who achieved clinical pregnancy and those who didn't.	123
36	Bar graph summarizing follicular recruitment parameters and oocyte/embryo outcomes in unexplained infertility women who achieved clinical pregnancy and those who didn't.	125

**Follicular Output RaTe (FORT) may predict Intra
Cytoplasmic Sperm Injection (ICSI) outcome**

Protocol of Thesis
Submitted for partial fulfillment of Master Degree in
Obstetrics & Gynecology

By
Omar Gamal Farag Mustafa
M.B.B.Ch., Faculty of Medicine, Ain Shams University,
2014
Obs. & Gyn. Registrar at Dar EL Shefa Hospital
(Ministry of Health Specialized Medical Centers)

Under Supervision of
Prof. Hesham Mohamed Fathy

Professor of Obstetrics and Gynaecology
Faculty of Medicine - Ain Shams University

Prof. Sherif Fathi El-Mekkawi

Professor of Obstetrics and Gynaecology
Faculty of Medicine - Ain Shams University

Dr. Ahmed Mohamed El-kotb

Lecturer of Obstetrics and Gynaecology
Faculty of Medicine - Ain Shams University

Faculty of Medicine
Ain Shams University
2017

Introduction

Ovarian stimulation is a key procedure necessary to achieve success in assisted reproductive techniques. Stimulation is achieved by the administration of exogenous gonadotropins in the form of Human Menopausal Gonadotrophin (HMG) to increase the follicular recruitment and oocyte yield. Confirmation of responsiveness of ovarian reservoir to FSH in terms of the development of the antral follicles is so far, a challenge for reproductive endocrinologists at clinics (**Shaheen, 2010**).

The appropriate response of antral follicles to FSH and a high-quality oocyte may result in a positive impact on outcomes of In Vitro Fertilization (IVF) and Intra Cytoplasmic Sperm Injection (ICSI). Poor response to (Controlled Ovarian Hyperstimulation) COH results in retrieval of few oocytes with reduced number of embryos available for transfer, leading to a decrease in pregnancy rates (**Rehman, 2013**).

Although the regulatory mechanisms determining the extent of sensitivity of individual antral follicles to FSH remain to be elucidated, adequate responsiveness to this glycoprotein presumably is characteristic of healthy and differentiated granulosa cells (**Shima, 1987**).

Indeed, granulosa cells displaying appropriate reactivity to FSH are not only endowed with functional FSH receptors but are also able to properly execute a cascade of specialized tasks as signal transduction, steroidogenesis and cell proliferation and differentiation. This physiological context suggests that responsiveness to FSH of antral follicles may constitute a marker of their health and reproductive competence and it leads us to hypothesize that patients endowed with a large proportion of FSH-responsive antral follicles should be more likely to become pregnant after assisted reproductive technologies (**Gougeon, 1996**).

The endocrinologists, thus, devised various methods to assess the ovarian reservoir and the expected responsiveness. Antral follicle count (AFC) is one of the non- invasive methods used for the assessment of the sensitivity of antral follicles to FSH (**Arslan, 2005**).

AFC represents the number of remaining primordial pool which corresponds to the number of oocytes retrieved; however, it does not influence the number of oocytes, embryo quality and the outcome of ICSI. The number of pre-ovulatory follicle count (PFC) obtained at the end of COH is estimated to be the best indicator of the number of retrieved oocytes (**Rehman, 2013**).

However, antral follicle count also includes the number of small antral follicles available before treatment. It means that follicular output rate (FORT) determines follicular response to exogenous HMG by the ratio of pre-ovulatory follicles to the existing pool of small antral follicles. The index has been investigated as an indicator of existing ovarian reservoir in response to stimulation and oocyte competence by a number of researchers like **Genro et al., 2011**, and **Gallot et al., 2012**.

Aim of the Work

The aim of the study is to assess the accuracy of the Follicular Output RaTe (FORT) as a predictor of pregnancy outcome after ICSI.

Question of the study:

In infertile women undergoing ICSI-ET treatment cycles; can Follicular Output RaTe (FORT) predict pregnancy outcome after ICSI accurately?

Hypothesis of the study:

In women undergoing ICSI-ET treatment cycles; Follicular Output RaTe (FORT) may predict pregnancy outcome after ICSI accurately.

Methods: participants, interventions and outcomes

Study design: Prospective cohort study.

Settings: The current study will be conducted in the Assisted Reproduction Unit (ART Unit) of Ain Shams University Maternity Hospital and a private IVF centre in the period between February 2017 and July 2018.

Population: A total of 400 infertile women will be enrolled in this study. Quota sampling will be used to recruit 100 infertile women undergoing IVF/ICSI in each of four study groups: PCOS group, tubal factor group, endometriosis group and unexplained infertility group.

RESEARCH METHODOLOGY

- After approval of the ethical committee of obstetric & gynecology department of Ain Shams University; all participants in the study will be given a written, informed consent, after explaining the details of the study to them.
- Participants ***included*** in this study will have the following criteria:
 - a. Age 18-39 years.
 - b. BMI (kg/m^2) of 18 – 29
 - c. Normal office hysteroscopy.