DEOXYRIBONUCLEIC ACID CYTOMETRIC ANALYSIS OF HUMAN GRANULOSA CELLS

Thesis
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بسم الله الرحمن الرحيم

" يا أيها الناس ضرب مثل فاستمعوا له إن الذين تدعون من
دون الله لن يخلقوا ذبابا ولو اجتمعوا له وإن يسلبهم الذباب
شيئا لا يستنقذوه منه ضعف الطالب والمطلوب
ما قدروا الله حق قدره إن الله لقوي عزيز "

« سورة الحج الآية ٧٢ – ٧٣ »

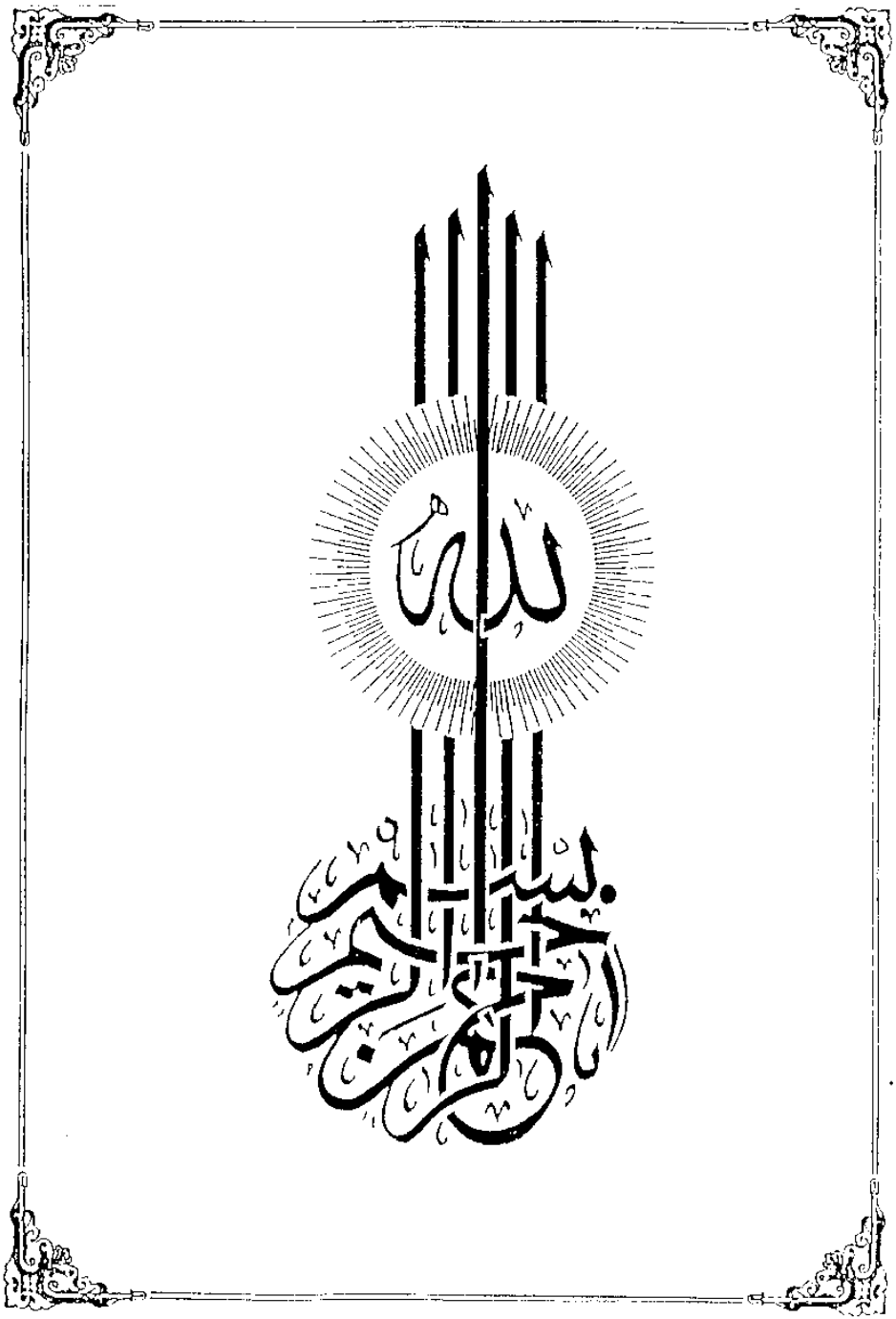
" هذا خلق الله فأروني ماذا خلق الذين من دونه بل الظالمون
في ضلال مبين "

« سورة لقمان الآية ١٠ »

" أفحسبتم أنما خلقناكم عبثا وأنكم إلينا لا ترجعون "

« سورة المؤمنون الآية ١١٥ »

صدق الله العظيم



"OMNE VIVUM EX OVO"

(all life from eggs)

William Harvey

De Generatione Aminalium (1651)

'He who see things grow from the beginning

will have the finest view of them"

Aristotle (300 B.C.)

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Finally, I thank those who taugh me what to do, and those who taugh me what not to do.

DEDICATION

TO THE MEMORY OF

MY MOTHER

LIST OF ABBREVIATIONS

A or $A\Delta^4$	Androstenedione
CC	Clomiphene Citrate
cAMP	Cyclic Adneosine Monophosphate
DNA	Deoxy Ribonucleic Acid
DHT	Dihydrotestosterone
E2	Estradiol
FSH	Follicle Stimulating Hormone
FF	Follicular Fluid
GV	Germinal Vesicle
GnRH	Gonadotropin - Relasing Hormone
GnRH.a	Gonadotropin - Releasing Hormone Analogue.
hCG	Human Chorionic Gonadotropin
hMG	Human menopausal Gonadotropin
IGF-I	Insulin-Like Growth Factor-I
IL	Interleukin
IVF-ET	In Vitro Fertilization - Embryo transfer
LH	Luteinizing Hormone
$\bar{x}$	Mean
OCCCs	Oocyte Cumulus Corona Cell Complexes
P	Progesterone
17(OH)Progesterone	17 Hydroxy Progesterone
S.D	Standard Deviation
T	Testosterone.
WHO	World Health Organization

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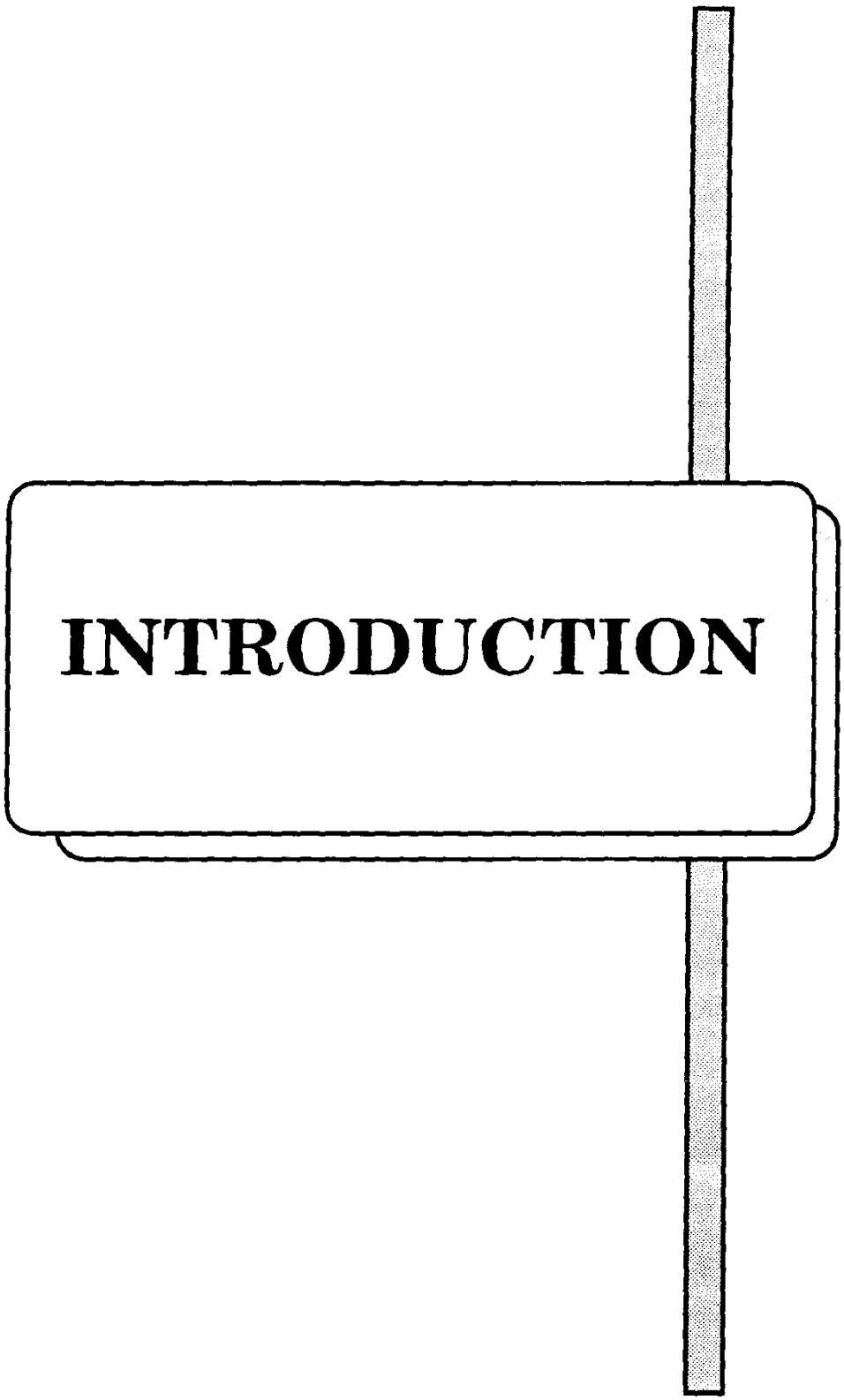

INTRODUCTION

Since the first report of a successful pregnancy after fertilization of human oocytes in vitro (**Edwards & Steptoe, 1979**), the proportion of oocytes successfully fertilized in vitro has increased (**Trounson et al, 1981; Edwards, 1981**). There remain however, a significant number of oocytes which either fail to undergo fertilization in vitro, or once fertilized, do not give rise to a successful pregnancy (**Carson et al, 1982**).

A major determinant of the success of In vitro fertilization is the selection of the optimal follicle containing an oocyte capable of being fertilized and producing a normal pregnancy (**Dlugi et al, 1985; Hill et al, 1989**).

Oocytes capable of undergoing successful fertilization and subsequently giving rise to pregnancy are thought to originate from the largest, most estrogenic, and by inference non-atretic follicle present at the time of mid-cycle gonadotropin surge (**Carson et al, 1982**). Follicular growth and oocyte maturation depend upon the constituents of the follicular fluid (**McNatty & Baird, 1978; McNatty et al, 1979(a)**, and follicular steroids may have either a stimulatory or an inhibitory effect on oocyte maturation (**Smith & Tenney, 1980**).

The classification of ovarian antral follicles as healthy or atretic is based on morphological criteria of the oocytes collected from them at the light microscopic level (**Edwards & Steptoe, 1975.; Veeck et al, 1983; Testart et al, 1983; Mohr & Trounson, 1984; Ben-Rafael et al, 1986; Veeck, 1986**).

In assessment of follicular well being, the hormonal composition

of the follicular fluid is taken into account together with the morphological criteria of the oocytes (**McNatty et al, 1979(a)**).

Baird & Fraser, 1974; Edwards et al, 1977; Bomsel-Helmreich et al, 1979; Edwards et al, 1980; Brailly et al, 1981; Hillier and De Zwart, 1981; Hillier et al, 1981; Kerin et al, 1981, showed that a healthy follicle is one with actively proliferating granulosa cells and a high level of estradiol in its follicular fluid, whereas an atretic follicle is one with low proliferation rate of granulosa cells, a low level of estradiol and a high level of androgen in its follicular fluid.

Westergaard et al, 1982 and Ben-Rafael et al, 1987(a) used cytometric DNA measurements on granulosa cells to distinguish healthy and atretic follicles and correlated their findings to the levels of follicular fluid steroids.

The use of DNA cytometry for assessment of follicular well being may offer some important advantages over other methods. **(Westergaard et al, 1982).** The technique represents a rapid method requiring only a small fraction of the entire cell population, thereby permitting the majority of the follicular aspirate to be immediately available for additional biochemical studies **(Westergaard et al, 1982).**

AIM OF THE WORK

AIM OF THE WORK

1- Studying the role of DNA image cytometric analysis of human granulosa cells in assessing oocyte maturation.

2- Studying the relations between granulosa cells proliferative indices, follicular fluid steroids (Estradiol, Progesterone and Androstenedione), oocyte maturity and fertilizability.

REVIEW OF LITERATURE