Effect of Danazol on Expression of Endometrial ανβ3 Integrin in Patients with Recurrent IVF-ET Failures: A Randomized Controlled trial

Thesis Submitted for partial fulfillment of MD in Obstetrics and Gynecology

By

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سورة طه الآيه رقم ۱۱۶



Acknowledgement

First of all, all gratitude is due to Allah almighty for blessing this work, until it has reached its end, as a part of his generous help, throughout my life.

Really I can hardly find the words to express my gratitude to **Dr.**Maged Ramadan Abou-Saeda, Professor of Obstetrics and Gynecology,
Faculty of Medicine, Ain Shams University, for his supervision, continuous
help, encouragement throughout this work and tremendous effort he has
done in the meticulous revision of the whole work. It is a great honor to
work under his guidance and supervision.

I would like to express my sincere appreciation and gratitude to **Dr.** Waleed Hitler El-Tantawy, Professor of Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University, for his continuous directions and support throughout the whole work.

I cannot forget the great help of **Dr. Kareem Mohammad Labib** Lecturer in Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University for his invaluable efforts and for his patience and support to get this work into light,

I cannot also forget the help of **Dr. Mohamed Abdelfattah Elsenity** Lecturer in Obstetrics and Gynecology, Faculty of medicine, Ain Shams University L **Dr. Mohammed Mahmoud Samy** Lecturer in Obstetrics and Gynecology Faculty of medicine, Ain Shams University for their great help L guidance to finish this work.

I am extremely sincere to my family who stood beside me throughout this work giving me their support.

Words fail to express my love, respect and appreciation to my wife for her unlimited help and support.

Lastly, all the love to my dear daughter for being patient, understanding and cheerful throughout this work.

Words are not enough to thank the Patients who shared in this study in order to achieve the best care for them.

Last but not least, I dedicate this work to my family and friends, whom without their sincere emotional support, pushing me forward this work would not have ever been completed.

Hamed Abdelsadek Aziz Hamed Al-Aarag

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List of Abbreviations

BBT : Basal body temperature

CAMP : Cyclic Adenosine Monophosphate

CAMs : Cell Adhesion Mollecules

CC : Clomiphene Citrate

COH : Contolled ovarian stimulation

COX : Cyclooxygenase

cPLA : Cytosolic Phospholipase A CSF : Colony Stimulating Factor

E : Eosin

ECM : Extracellular matrix components

EDPA : Intraendometrial power Doppler area.

EEC : Endometrial Epithelial cells

ELISA : Enzyme-linked immunosorbent assay

ER : Estrogen receptor ER : Estrogen receptor

ESC : Endometrial Stromal Cells

ET : Embryo transfer

FAK : Focal adhesion kinase

FN : Fibronectin

FSH : Follicle Stimulation Hormone

H : Hematoxilin

HG-EGF : Heparin binding epidermal growth factor

HOXA : Homeobox gene

HSG : Human Chorionic Gonadotrophin

HSG : Hysterosalpingography

ICAM-1 : Intercellular adhesion molecules 1ICSI : Intracytoplasmic Sperm Injection

IGF : Insulin like growth factorIHC : Immunohistochemistry

IL : Interlukin

IUI : Intrauterine Insemination

IVF : In Vitro fertilization

List of Abbreviations (Cont.)

LH : Luteinizing Hormone

LIF : Leukemia inhibiting factor

LTs : Leukotrienes

MAP : Mitogen-activated proteinMCP : Membrane Co-factor proteinMMPs : Matrix metalloproteinases

MUC : Mucin

OPN : Osteopontin

PCOs : Polcystic ovary syndrome

PCT : Post coital test PG12 : Prostacyclin

PGS : Prostaglandin Synthase

PGs : Prostaglandins

PGT : Prostaglandin transporter
PR : Progesterone receptor
RPL : Recurrent Pregnancy loss
TGF : Transforming growth factor
TNF-a : Tumor Necrosis Factor a

TXa : Thromboxanes

VEGF : Vascular endothelial growth factor

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ABSTRACT

Introduction: The endometrium remodeles throughout the menstrual cycle, and exhibits only a short period of receptivity, known as the "window of implantation". The endometrium becomes receptive to blastocyst 6-8 days after ovulation and remains receptive for 4 days (cycle days 20-24). Failed implantation remains a significant cause of reproductive failure in both spontaneous and assisted reproduction cycles. Aim of the work: The aim of this study is to determine the effect of Danazol on expression of endometrial ανβ₃ integrin in patient with recurrent IVF-ET failures. Patients and Methods: Randomized controlled trial; The current study was conducted at Ain Shams University Maternity Hospital during the period between August 2015 and January 2017; A total of 30 women with previous failed IVF/ICSI trials were included in this study. Results: The current study was conducted at Ain Shams University Maternity Hospital during the period between August 2015 and January 2017. A total of 30 women with previous failed IVF/ICSI trials were included in the study. Conclusion: From this study we concluded that, treatment of patients with recurrent implantation failure with Danazol increases the expression of endometrial αvβ3 integrin which makes the endometrium more receptable for the implanting embryo, thus increases the pregnancy rate.

Key words: CAMP: Cyclic Adenosine Monophosphate; CAMs: Cell Adhesion Mollecules; CC:Clomiphene Citrate; COH: Contolled ovarian stimulation; COX:Cyclooxygenase; cPLA:Cytosolic Phospholipase A

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Introduction

The endometrium remodeles throughout the menstrual cycle, and exhibits only a short period of receptivity, known as the "window of implantation" (*Croxatto et al.*,1987).

The endometrium becomes receptive to blastocyst 6-8 days after ovulation and remains receptive for 4 days (cycle days 20-24) (*Bergh et al.*,1992).

Failed implantation remains a significant cause of reproductive failure in both spontaneous and assisted reproduction cycles (*Smith et al.*,1998).

The implantation is a complex procedure that can be divided into three distinct steps: opposition, attachment, and invasion (*Norwitz et al.*,2001). Shortly after the opposition step, an integrin-dependent adhesion occurs. This allows the blastocyst to attach firmly to the uterine wall and trophoblasts transmigrate across the luminal epithelium, burying the embryo beneath the uterine wall (*Russell et al.*,2007).

To achieve implantation, many molecules (hormones, cytokines, integrins, enzymes, etc) involve in the dialogue between the human blastocyst and the maternal endometrium (*Valles et al.*,2006).

Integrins are cell-surface adhesion receptors that play key role in mediating numerous physiological processes, including inflammation, migration, adhesion, and proliferation (*Borthwick et al.*,2003).

Integrins composed of an alpha and a beta subunit. Each subunit comprises an extracellular domain, a transmembrane region and an intracellular domain (*Singh et al.*,2009).

Integrins serve as receptors for components of extra cellular matrix such as osteopontin, fibronectin and collagens. These components have the capacity to act as bridging molecules between the blastocyst and the endometrial surface during the adhesion phase of the implantation process (*Tabibzadeh et al.*,1999, *Campbell et al.*,1995, *Johnson et al.*,1999, *Johnson et al.*,2003).

The role of integrins in implantation has been widely reviewed (*Lessey et al.*,2002, *Aplin et al.*, 2004, *Nardo et al.*,2002, *Kimber et al.*,2000). The extensive work of *Lessey et al* showed that three integrins ($\alpha_1\beta_1$, $\alpha_4\beta_1$, and $\alpha\nu\beta_3$) express in uterine epithelium during implantation window (*Lessey et al.*, 2000, 2002).

In other studies, it was reported that the best characterized cell adhesion molecules on the luminal surface of the endometrium are $\alpha v \beta_3$ integrin and its ligand osteopontin, repeatedly found in genome-wide studies of human receptive endometrium (*Casals et al.*,2010, *Borthwick et al.*,2003, *Carson et al.*,2002, *Riesewijk et al.*,2003). Blocking $\alpha v \beta_3$ interactions in mouse or rabbit models impairs implantation (*Illera et al.*,2000, 2003).

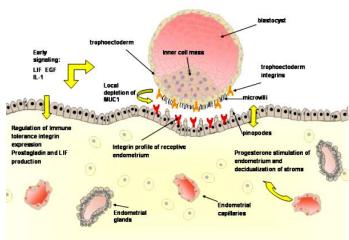


Fig. (1): A schematic representation of a blastocyst approaching the receptive endometrium, defined by the integrin profile and appearance of pinopodes. Early signaling between the blastocyst and the endometrium precedes the attachment (*Staun-Ram and Shalev*, 2005).

Establishment of an effective treatment for the implantation failure is the most important theme in assisted reproductive technology (ART). The efficacy of Danazol treatment for the repeated failure of IVF-ET with morphologically normal embryos has been previously reported (*Tei et al.*,1998).

Danazol, an isoxasol derivative of $17 \, \alpha$ -ethinyltestosterone, has been widely used to treat patients with endometriosis and adenomyosis. In addition to its hormonal activity, Danazol is also known to have a variety of immunoregulatory effects on the eutopic endometrium (*Dmowski et al.*,1988, *Hill et al.*,1987).

However, the biological effect of Danazol on endometrial receptivity has not been extensively studied. It also prompted us to examine the effect of Danazol on endometrial integrin $\alpha v \beta_3$ expression. Here we