Effect of Danazol on Expression of Endometrial αvβ3 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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﴿وَقُل رَّبِّ زِدْنِي عِلْماً﴾

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Hamed Abdelsadek Aziz Hamed Al-Aarag

Contents

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 1
ICSI	:	Intracytoplasmic Sperm Injection
IGF	:	Insulin like growth factor
IHC	:	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 protein
MCP	:	Membrane Co-factor protein
MMPs	:	Matrix metalloproteinases
MUC	:	Mucin
OPN	:	Osteopontin
PCOs	:	Polcystic ovary syndrome
PCT	:	Post coital test
PGl2	:	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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Effect of Danazol on Expression of Endometrial αvβ3 Integrin in Patients with Recurrent IVF-ET Failures: A Randomized Controlled trial

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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 $\alpha v\beta_3$ 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 $\alpha v\beta_3$) 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)***.

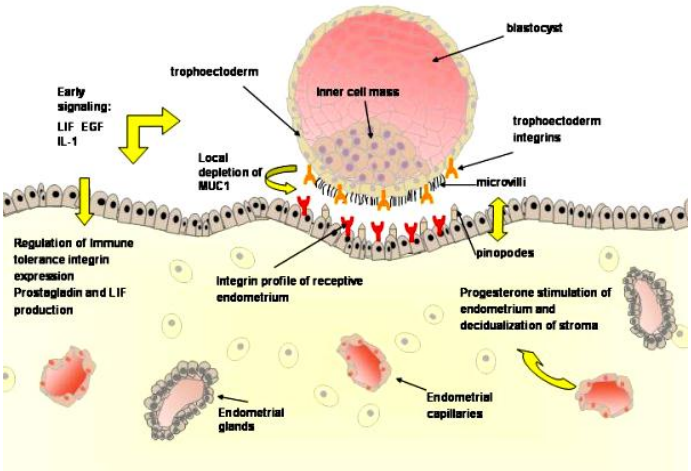

Fig. (1): A schematic representation of a blastocyst approaching the receptive endometrium, defined by the integrin profile and appearance of pinopodes. Early signaling between the blastocyst and the endometrium precedes the attachment ***(Staun-Ram and Shalev, 2005).***

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 α-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