Effect of Endometrial Injury on Pregnancy Rate in Women with Unexplained Infertility in Cycles Stimulated by Letrozole: Randomized Controlled Trial

Thesis

Submitted For Partial Fulfillment of Master Degree In Obstetrics and Gynaecology

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Wesam Abd El Hameed

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List of abbreviations

APAs	**:**	Antiphospholipid Antibodies
ART	**:**	Assisted reproductive technologies
ASRM	**:**	American Society for Reproductive Medicine
BMI	**:**	Body mass index
Ca	**:**	Calcium
Cad	**:**	Cadherin
CAM	**:**	Cellular adhesion molecules
cAMP	**:**	Cyclic adenosine monophosphate
CC	**:**	Clomiphene citrate
COH	**:**	Controlled ovarian hyperstimulation
CPR	**:**	Clinical Pregnancy rate
CSF	**:**	Colony-stimulating factor
DNA	**:**	Deoxyribonucleic acid
E2	**:**	Estradiol
ECM	**:**	ExtracellularMatrix
EEC	**:**	Endometrial epithelial cells
EGF	**:**	Epidermal growth factor
ER	**:**	Estrogen receptor
ERA	**:**	Endometrial Receptivity Array
ESHRE	**:**	European Society for Human Reproduction and Embryology
ET	**:**	Embryo transfer
FET	**:**	Frozen embryo transfer
FSH	**:**	Follicle-stimulating hormone
GnRH	**:**	Gonadotropin releasing hormone

List of abbreviations (Cont.)

HB-EGF	**:**	Heparin-binding EGF-like growth factor
HCG	**:**	Human chorionic gonadotropins
hCG	**:**	Human chorionic gonadotropin
HMG	**:**	Human menopausal gonadotropin
HSG	**:**	Hysterosalpingography
ICI	**:**	Intracervical insemination
ICSI	**:**	Intracytoplasmic sperm injection
IGFBP	**:**	Insulin growth factor binding protein
IGFs	**:**	Insulin growth factors
IL-1	**:**	Interlukein-1
InhA	**:**	InhibinA
InhB	**:**	InhibinB
IUI	**:**	Intrauterine insemination
IVF	**:**	In vitro fertilization
IVF-ET	**:**	in-vitro fertilization–embryo transfer
LH	**:**	Luteinizing hormone
LIF	**:**	Leukemia inhibitory factor
LPD	**:**	Luteal phase defect
MMPs	**:**	Matrix metalloproteinass
mRNA	**:**	Messenger ribonucleic acid
MUC1	**:**	Mucin1
NICE	**:**	National Institute of Clinical Excellence
NIH	**:**	National Institutes of Health
P	**:**	Probability value
PBMC	**:**	peripheral blood mononuclear cells
PET	**:**	Personalized embryo transfer

List of abbreviations (Cont.)

PI	**:**	Pulsatility index
PR	**:**	Pregnancy rate
RCT	**:**	Randomized controlled trial
RI	**:**	Resistance index
RIF	**:**	Recurrent implantation failure
RNA	**:**	Ribonucleic acid
SEM	**:**	Scanning electron microscopy
TGF- β	**:**	Transforming growth factor-β
TI	**:**	Timed intercourse
TNF-alpha	**:**	Tumour necrosis factor alpha
UI	**:**	unexplained infertility
US	**:**	Ultrasonography
VTRL		Variable number of identical tandem repeat
WHO	**:**	World Health Organization
ZP	**:**	Zonapellucida

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Introduction

Infertility is a continuous challenge for all gynecologists worldwide. Infertility is defined as the failure to achieve pregnancy after a year (in women less than 35 years old) or 6 months (in women more than 35 years old) of unprotected sexual intercourse. Studies show that infertility affects about 15 to 20 percent of couples worldwide ***(Evers, 2002)***.

The causes of infertility are due to female factors, male factors, or combination of both or due to unknown causes. The common causes are ovulatory disorders or anovulation, tubal factors, abnormal sperm parameters, endometriosis, and uterine or cervical factor ***(Collins et al., 1995)***.

Less common causes are disturbance of immunological balance, genetic defects and reproductive physiology ***(Pellicer et al., 1995).***

Unexplained infertility is a term which has been applied to 10-15% of infertile couples after diagnostic workup ***(Smith et al., 2003)***.

Implantation requires complex sequence of signaling events between receptive endometrium, normal and functional embryo occurs 6-7 days after fertilization ***(Aplin et al., 2000).***

Embryo implantation is the most critical step in the reproductive process. It happens through a unique biological phenomenon, as the blastocyst becomes intimately connected to the endometrial surface ***(Denker, 1993).***

Implantation failure remains unsolved problem in reproductive medicine and is considered as a major cause of infertility in otherwise healthy women. It may be due to abnormal uterine receptivity which is difficult to diagnose and treat ***(Margalioth et al., 2006).***

Many useful modalities include diagnostic and operative hysteroscopy to identify and correct anatomical defects in the uterus, administrating medications to induce endometrial proliferation, administering drugs that affect immune system and more recently, intentional endometrial injury through biopsy (scratch) and or curettage ***(Bosteels et al., 2010)***.

Barash et al. (2003) explored that local endometrial injury in the cycle before IVF treatment increases the success rate of implantation in patients who failed to conceive after one or more IVF treatment cycle. Similarly, a further study identified favorable influence of local endometrial injury in ICSI patients with high order implantation failure ***(Raziel, et al., 2007)***.

Because of the difference of the method used for including endometrial injury and timing in relation to ovarian stimulation, there is considerable heterogenecity in the studies ***(Zhou et al., 2008).***

Aromatase inhibitors have been used successfully to induce ovulation in women with the polycystic ovary syndrome.In addition; multiple reports suggest that aromatase inhibitors may be effective alternative agents for ovarian stimulation in couples with unexplained infertility ***(Al-Fozan et al., 2004).***

Although the underlying mechanism of endometrial injury remains unknown. Hypotheses include: that it might induce decidualization, increasing chance of implantation; it increases the secretion of cytokines, interleukins, growth factors, macrophages and dendritic cells which might be important to embryo implantation and that endometrial scratching might lead to better synchronicity between the endometrium and the embryo ***(MorG and Kogak, 2008).***

Aim of the Study

The objective of this randomized controlled trial (RCT) was to determine whether endometrial injury (scratching) could improve the probability of pregnancy in a group of women with unexplained infertility in cycles stimulated by letrozole.

Research question

Will endometrial scratch increase the probability of pregnancy rate in Couples with unexplained infertility in cycles stimulated by letrozole?.

Research hypothesis

- Alternative hypothesis: endometrial scratching leads to higher clinical pregnancy rates in women with unexplained infertility in cycles stimulated by letrozole.

Outcome measures

- **Primary outcome:** clinical pregnancy rate, defined as(the presence of at least one gestational sac on ultrasound at 6-7 weeks).

- **Secondary outcome:** Chemical pregnancy rate (evidenced by positive pregnancy test in serum or urine without ultrasound evidence of a gestational sac), first trimester miscarriage rate, multiple pregnancy rate defined as (a pregnancy with more than one gestational sac detected by transvaginal sonography at 6-7 weeks of gestation).

Patient and Methods

Study design: randomized controlled trial.

Settings: infertility outpatient clinic in Ain Shams University Maternity Hospital .

Population: The study will include 350 women with unexplained infertility.

- Participants ***included*** in this study will have the following criteria:

- Age between 20-35 years.
- Unexplained infertility defined as the absence of a definable cause for a couple's failure to achieve pregnancy after 12 months of attempting conception despite a thorough evaluation, or after six months in women 35 years old and older [Moghissi, Wallach 1983, 2008]. Authorities vary in their concept of what constitutes a thorough evaluation, and these opinions have evolved over time. Currently, a thorough evaluation typically includes documentation of: ovulation ,tubal patency.
- Normal transvaginal ultrasonography
- Normal hysterosalpingography and/or diagnostic laparoscopy.
- Regular ovulation confirmed by mid-luteal progesterone
- Absence of any structural pathological findings in laparoscopy.
- Normal parameters of male semen analysis according to WHO criteria 2010.
- Written and signed informed consent by the patient to participate in the study.

The following patients will be ***excluded*** from the study:

- Age more than 35 years.

- Abnormal ultrasonographic finding, e.g. endometrial polyps, fibroids or ovarian cysts.
- Abnormal hysterosalpingographic finding, e.g. hydrosalpinx or peritoneal adhesions.
- Abnormal endocrinological profile, e.g. hyperprolactinemia.
- Abnormal semen analysis parameters according to WHO criteria 2010.
- Chronic medical disorder, e.g. hypertension, autoimmune disorders, DM.
- Mental condition rendering the patients unable to understand the nature, scope and possible consequences of the study.

Sample size justification:

Assuming that pregnancy rate in women undergoing local endometrial injury (LE) was 14.9% compared with 5.8% in controls not subjected to LEI ***(Parsanezhad et al., 2013).***

It is estimated that a sample size of 175patints in either study group (Total 350 patients) would achieve of 80% (type 2 errors, 0.2) to detect a statistically significant difference between the 2 groups with a confidence level of 95% (type 1 error, 0.05) using two-sided Z test with pooled variance. The pregnancy rate is assumed to equal 5.8%in both groups under the null hypothesis and to equal 14.9% or 5.8%in the endometrial scratch or control group, respectively, under the alternative hypothesis.

Research Methodology

- After approval of the ethical committee, all participants in the study will give a written, informed consent, after explaining the details of the study to them.

- ***Randomization:***

Patients fulfilling the inclusion criteria will be randomized to two groups.

Study Group:

This group will include 175 women with unexplained infertility. This group will receive letrozole and undergo endometrial scratching.

Control Group:

This group will include 175 women with unexplained infertility. This group will receive letrozole .

Methods/design

All the patients received letrozole tab 5mg/day for 5 consecutive days, with therapy initiated on cycle day 3. When the dominant follicles reached 18-22 mm, as measured by transvaginal ultrasound, HCG (Choriomon® 5000 IU/amp, IBSA, Switzerland)) was injected intramuscularly, in a dose of 5,000-10,000 IU, and timed intercourse was advised, 36 h after HCG injection and the days after. Vaginal ultrasound was done about 60 h after HCG injection to confirm follicular rupure. Also on day 4-7, but only for the study group patients, local endometrial injury was performed. B-hCG was checked if the patient experienced one week missed period.

Technique of endometrial injury:

Endometrial injury was done only for patient of the study group. It was done on day 4-7, under complete aseptic conditions, no anesthesia, was given to cases. Endometrial scratching was performed on the posterior wall, midline, using pipelle endometrial sampling (Pipelle; Genetics Medical Products, Hamont-Achel, Belgium).

- All subjects will be subjected to the following:

a. Proper history taking, examination and investigations to:
   - Diagnose the cause of infertility
   - Ensure fitting the definition of “unexplained infertility”