Comparative Study Between The Effect of Heparin And Nitric Oxide on Uterine Blood Flow And Implantation Rates At Assisted Reproductive Techniques (ART)

Thesis

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List of Abbreviations

ADP:	Adenosine diphosphate
ART:	Assisted reproductive technology
bFGF:	Basic fibroblast growth factor
CNOS:	Constitutive nitric oxide synthase
CO:	Carbon monoxide
COH:	Controlled ovarian hyper-stimulation
CSF:	Colony stimulating factor
ECM:	Extracellular matrix
EGF:	Epidermal growth factor
eNOS:	Endothelial nitric oxide synthesis
ER:	Estrogen receptor
FSH:	Follicle stimulating hormone
GIFT:	Gamete intrafallopian transfer
GMP:	Guanizine monophaosphate
GnRHa:	Gonadotrophin releasing hormone agonist
GTN:	Glyceryl trinitrate
GV:	Graffian follicle
HB-EGF:	Heparin binding epidermal growth factor
HCG:	Human chronic gonadotrophin
HIV:	Human immunodeficiency virus
HMG:	Human menopausal gonadotrophin
HPV:	Hypoxic pulmonary vasoconstriction
HS:	Heparan sulfate
ICSI:	Intra cystoplasmic sperm injection
IGF:	Insulin growth factor
IGFBP:	Insulin like growth factor binding protein
IL:	Interleukin
iNOS:	Inducible nitric oxide synthase
IVF:	in vitro fertilization

LE: Luminal epithelium
LH: Lutinizing hormone
LIF: Leukemia inhibitory factor
MAG: Mouse ascites golgi
MMP: Matrix metalloproteinase
mRNA: Messenger RNA
MUC: Mucin
NAC: N-acetyl cysteine
NANC: Non adrenergic non cholinergic
NO: Nitric oxide
NOS: Nitric acid synthase
OCC: Oocyte cumulus complex
OHSS: Ovarian hyperstimulation syndrome
PAI: Plasminogen activator inhibitor
PCOS: Polycystic ovary syndrome
PGE2: Prostaglordin E2
PGF2: Prostaglandin F2
PI: Pulsatility index
PID: Pelvic inflammatory disease
PR: Progestron receptor
PVP: Poly vinyl pyrrolidene
PVS: Pervitelline space
PZD: Partial zona dissection
SNP: Sodium nitropresside
SUZI: Subzonal sperm injection
TE: Trophectoderm
TGF: Transforming growth factor
TIMP: Tissue inhibitors of metallo-proteinases
TXA2: Thromboxane A2
ZIFT: Zygot intrafallopian transfer

INTRODUCTION

Fertilization of the human oocyte by human sperm in vivo requires the interaction of capacitated spermatozoa with ovulated oocytes, most often within the ampullary portion of the fallopian tube. Capacitation of sperm takes place in the female reproductive tract and involves both changes in sperm motility and changes in the sperm cell membrane that allow the acrosome reaction. Acrosome-reacted sperms are able to penetrate the oocyte's cumulus oophrus and zona pellucida, binding to the oocyte cell membrane and promoting fertilization. The interaction of spermatozoa and oocyte is not a chance occurrence, as complex oocyte-sperm intercommunications appear to play an important role in this process. The mandate of assisted reproductive technology (ART) is to attempt to re-create precisely those processes known to occur in unassisted conception *(Yao & Schust, 2002)*.

Since the birth of Louise Browen in July 1978 & the birth of the first ICSI child in January 1992 many couples with longstanding female factor or male factor infertility can be helped to overcome their infertility resulting in a birth of a child *(Van-steirteghem et al., 2002)*.

ICSI in now widely available in large number of assisted conception units internationally and has revolutionized the management of male factor infertility *(Meniru, 2001)*.

During the past two decades, ART have revolutionized the treatment of infertility. ART now accounts for between 1% and 3% of annual births in many western countries and IVF services are growing world wide *(Lamb and Lipshult, 2003)*.

One area in which a breakthrough still has not occurred is the mechanism of embryo implantation. The incidence of birth per embryo replaced in IVF still is considerably lower than that of fertile couples who conceive naturally. Despite the major advances in fertilization rate implantation rates after embryo replacement remain low *(Lamb and Lipshult, 2003)*.

There is no simple, cheap and non-invasive method that can adequately predict the outcome of ART. Uterine receptivity seems to be of great importance for achievement of normal pregnancy during an ART program but it is likely to be regulated by a number of factors, many of which are still obscure *(Carbillon et al., 2001)*.

The assessment of uterine artery flow by Doppler examination is easy and reproducible and high impedence at the end of the follicular phase is a good predictive indicator of poor endometrial receptivity (*Carbillon et al., 2001*).

Other studies show that neither endometrial thickness nor endometrial blood flow appeared to correlate with pregnancy rate *(Chien et al., 2002)*.

Nitric oxide (NO) is a major paracrine mediator of various biological processes, including vascular functions and inflammation. In the human endometrium, nitric oxide synthase have been localized in the glandular epithelium and in endometrial microvascular endothelium, primarily during the luteal phase **(Chwalisz and Garfield, 2000)**.

Nitric oxide (NO) has been shown to play an important role during advanced gestation, although its role during early pregnancy is unclear ***(Castro et al., 2002)***.

The interaction of the epidermal growth factor EGF) receptor with its ligand, heparin-binding EGF like growth factor (HB-EGF), is considered important for maternal-embryonic dialogue during blastocyst implantation and trophoblast invasion **(Yoo et al., 1997)**.

Activation of EGF receptor influences blastocyst implantation as well as the subsequent behavior of trophoblast cells during placentation **(Raab et al., 1996)**.

Recent evidence indicates that HB-EGF is expressed in the human uterus during the earliest stages of pregnancy. HB-EGF expression is maximal during the late secretory phase, when the human endometrium becomes receptive for blastocyst implantation ***(Micholsky et al., 2002)***.

AIM OF THE WORK

This is a prospective randomized controlled comparative study aiming to evaluate the use of heparin versus nitric oxide donors would affect the uterine blood flow and implantation rate in women under-going Assisted Reproductive Techniques (ART).

ASSISTED REPRODUCTIVE TECHNOLOGY

Assisted reproductive technology (ART) has become the frontier of both infertility treatment and research. Nowadays, ART is widespread all over the world and become part of treatment modalities in infertile couples. Beyond practical debates ART also presents moral and sometimes ethical dilemmas. Society should be alert so those advances in laboratory techniques do not lead to deviant interference in the process of life creation just to satisfy scientific curiosity *(Ron et al., 1993).*

The first birth in 1978 following IVF treatment by Steptoe and Edwards marked the beginning of a rapid expansion of treatment modalities available to infertile couples as well as an improvement in success rates. IVF and many other assisted conception treatments are now routinely carried out in clinical practice and are proving to be more efficient and cost effective than some traditional remedies, such as tubal surgery *(Meniru, 2001).*

Definition:

Assisted reproductive technology (ART) refers to all techniques involving direct retrieval of oocytes from the ovary. The first and still most common procedure is invitro

fertilization, but there is an ever-increasing list of technologies.

Table (1): Sowing types of ART procedures.

IVF	Invitro fertilization: extraction of oocytes. fertilization in the laboratory, transcervical transfer of embryos into the uterus.
GIFT	Gamete intrafallopian transfer: the placement of oocytes and sperm into the fallopian tube.
ZIFT	Zygot intrafallopian transfer: the placement of fertilized oocytes into the fallopian tube.
TET	Tubal embryo transfer: the placement of cleaving embryos into the fallopian tube
POST	Peritoneal oocyte and sperm transfer: the placement of oocytes and sperm into the pelvic cavity.
In addition, techniques of sperm retrieval and sperm injection are now part of the assisted reproductive technology armamentarium:	
ICSI	Intracytoplasmic sperm injection (or a single spermato-zoon).
TESE	Testicular sperm extraction.
MESA	Microsurgical epididymal sperm aspiration

(Sauer et al., 1995).

Indication of ART:

Originally ART was developed for patients with absent or irreparably damaged fallopian tubes, as ART evolved and become more commonly available in indications widened currently it is performed in women with infertility due to such disorders as unexplained infertility, immunologic infertility, male factor infertility and endometriosis. The minimal requirements are that patient have normal uterine cavity, a source of oocytes, and enough sperm to achieve fertilization *(Laufer et al., 1989).*

1. Tubal factor:

Tubal diseases include Tubal block both proximally and distally, peritubal adhesion and hydrosalpinx *(Nackley and Muasher, 1998)* due to any of this causes:

- Infections either ascending from lower genital tract pelvic infection especially chlamydia PID "Pelvic inflammatory disease" → Tubal block or peritubal adhesion and decrease motility of the tubes.
- In cases of salpingectomy due to ectopic pregnancy.
- Adhesion after abdominal or pelvic surgery → Peritubal adhesion and decrease oocyte pick up mechanisms.

(Rebar, 1998).

2. Unexplained infertility:

Patients with unexplained infertility comprise a heterogeneous group who may have defects in sperm or oocyte function, fertilization, embryo development, fallopian tube function, or embryo implantation. The determination that undetectable factors are the cause of a couple's infertility assumes that a thorough evaluation of reproductive potential has been completed on both partners. There is general agreement in the medical literature ***(Shushan et al., 1995)*** that the investigation should include a demonstration of the following:

1. Regular ovulatory cycles with appropriate corpus luteum steroidogenesis as documented by hormones analyses and ultrasonography with or without endometrial biopsy.

2. Normal fallopian tube and uterine anatomy based on hysterosalpingography with or without laparoscopy/ hysteroscopy.

3. Absence of pelvic factors such as endometriosis and adhesive disease.

4. Normal seminal fluid analysis.

Assisted reproductive technologies (ART) are recommended if spontaneous conception has not occurred within a 3-year period of unprotected intercourse.

Aggressive treatments should be undertaken sooner in women aged 35 years or older and in those with decreased ovarian reserve as documented by an elevation in serum follicle-stimulating hormone (FSH) on cycle day 3 or an abnormal clomiphere citrate challenge test ***(Hesla and Schoolcraft, 1997).***

Management of unexplained infertility generally is empiric, controlled ovarian stimulation and intrauterine insemination has been proposed as an effective method of treatment. Gamete Intra-fallopian transfer also has been tried successfully in the management of unexplained infertility. Invitro fertilization (IVF) with embryo transfer has been reported to produce good pregnancy rate among Patient with unexplained infertility ***(Aboulghar et al., 1999).***

3. Male factor:

IVF has been an accepted treatment for male factor infertility when it does not respond to other therapies ***(Speroff et al., 1994).*** As relatively few sperms are required to effect fertilization invitro compared to invivo the application of IVF to treat couples in which the only etiology for infertility is a male factor, seems logical ***Quigley et al., 1984; Hirsch et al., 1986).***

Table (2): Show the most common causes of male infertility and their distribution.

Male factor problem	Percentage
No demonstrable cause (normal semen and sexual /ejaculatory function	48.5
Idiopathic abnormal semen (no cause for abnormal semen parameters	26.4
Varicocoele	12.3
Infectious factors	6.6
Immunological factors	3.1
Other acquired factors	2.6
Congenital factors	2.1
Sexual factors	1.7
Endocrine disturbances	0.6

Source: Adapted from ***Farley and Belsey (1988).***

4. Endometiosis:

Endometriosis was recorded as a diagnosis if it was noted laparoscopically (all stages of endometriosis from minimal to sever were included) ***(Miller et al., 1999)*** endometiosis causes infertility by any of this means:

- Induce pelvic adhesions → decrease tubal motility.
- Endometriotic tissue produces toxic compounds that can interfere with the interaction between the spermatozoa and oocyte.

- Endometriosis can interfere with ovulation in 10% of patients.
- Change position of uterus by adhesion.

(Kovacs, 1997).

5. Immunological factor:

The presence of antisperm antibodies in either the female or the male can interfere with the process of conception. In the female, antisperm antibodies have been detected in the plasma, genital tract and follicular fluid. In males antisperm antibodies can be present in plasma and semen *(Bronson et al., 1984).*

These antisperm antibodies can be produced when there is damage to the testes, infection or obstruction to the transport of spermatozoa along the male genital tract. Antisperm antibodies may bind to spermatozoa and prevent them from moving, also they may prevent the fertilization of the egg by affected spermatozoa *(Rowe et al., 2000).*

5. Hostile cervical mucus:

Mucus may be regarded as "hostile" where it is resistant to spermatozoal invasion through a barrier or spermicidal effect that seems inherent in the mucus itself, with all other parameters of both partners seemingly normal. Cervical mucus is said to be hostile if it is acidic,