Effect of Intrauterine Injection of Human Chorionic Gonadotropin at the Day of Ovum Pick-Up on Patients Undergoing ICSI for Recurrent Implantation Failure

Thesis

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List of Abbreviations

AFC : Antral follicle count
ART : Assisted reproductive technology
ASRM : American society for reproductive medicine
$E_{2:}$: Estradiol
ESHRE : European society of human reproduction and embryology
ET : Embryo transfer
FET : Frozen embryo transfers
FSH : Follicle stimulating hormone
G-CSF : Granulocte colony stimulating factor
GnRH : Gonadotropin releasing hormone
hCG : Human chorionic gonadotropin
HLA : Human leukocyte antigen
HMG : Human menopausal gonadotropin
ICSI : Intracytoplasmic sperm injection
IGFBP-1 : Insulin like growth factor binding protein-1
IL-1 R : Interleukin-1 receptor
IMSI : Intracytoplasmic morphologically selected sperm injection
IUI : Intrauterine insemination
IVF : In vitro fertilization
LH : Luteinizing hormone
LIF : Leukemia inhibitory factor
LMP : Last menstrual period
M II : Metaphase II
M-CSF : Macrophage colony stimulating factor
MMP-9 : Matrix metallopeptidase-9
NK : Natural killer

List of Abbreviations *(Cont.)*

OPU : Ovum pick-up
PB : Polar body
PGD : Preimplantation genetic diagnosis
PGS : Preimplantation genetic screening
PN : Pronucleus
PPARs : Peroxisome proliferator activated receptors
PVP : Polyvinylrrolidone
rFSH : Recombinant follicle stimulating hormone
r-hLIF : Recombinant human leukemia inhibitory factor
RIF : Recurrent implantation failure
TH : T-helper cells
TSH : Thyroid stimulating hormone
TVUS : Trans-vaginal ultrasound
VEGF : Vascular endothelial growth factor
ZP : Zona pellucida

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ABSTRACT

Background: Implantation is a complex initial step in the establishment of a successful pregnancy. Although embryo quality is an important determinant of implantation, temporally coordinated differentiation of endometrial cells to attain uterine receptivity and a synchronized dialog between maternal and embryonic tissues are crucial. **Aim:** The aim of the present study is to investigate the value of intrauterine injection of hCG at day of ovum pick-up in patients undergoing intracytoplasmic sperm injection in an attempt to improve implantation and pregnancy rates on patients with recurrent implantation failure. **Patients and Methods:** The recent study was designed as a prospective case-control, randomized, single blinded study, from February 2015 to January 2016, conducted at ART unit of Ain Shams University, maternity hospital. One hundred participants were assigned into the study, who were prepared to undergo an ICSI trial after a failed previous cycle or more. Patients were divided in to two groups: Group (1): The study group; included 50 patients who were injected by 500 IU of hCG intrauterine at the day of ovum pick-up. Group (2): The control group; included 50 patients, age matched, who went through the study without intrauterine injection. **Results:** The current study revealed that: **Conclusion:** it was found that this approach significantly improved pregnancy and implantation rates, with even improvement of the endometrial thickness at the day of embryo transfer; however miscarriage rates were non-significant between both groups.

Key words: Human chorionic gonadotropin, Ovum pick-up, Recurrent Implantation Failure

Introduction

Implantation is a complex initial step in the establishment of a successful pregnancy. Although embryo quality is an important determinant of implantation, temporally coordinated differentiation of endometrial cells to attain uterine receptivity and a synchronized dialog between maternal and embryonic tissues are crucial. The exact mechanism of implantation failure is still poorly understood ***(Cakman and Taylor, 2010).***

Successful implantation after in vitro fertilization (IVF) and embryo transfer (ET) depends on various factors related to the embryo quality and the endometrial receptivity. It is important that the embryo reaches the endometrial cavity during the period in which the endometrium is receptive, known as the ‘‘implantation window’’ ***(Psychoyos, 1986).***

It is estimated that about 50% to 75% of lost pregnancies are due to failure of implantation ***(Norwitz et al., 2001).***

Major advances have occurred in clinical and laboratory IVF techniques in recent years; however, the pregnancy rates remain around 30% per cycle ***(de Mouzon et al., 2002).***

Implantation is a very intricate process that is regulated by many factors, one of the most important of which is human chorionic gonadotropin (hCG) ***(Tsampalas et al., 2010).***

Human chorionic gonadotropin (hCG) is a heterodimeric placental glycoprotein hormone that is required to maintain pregnancy. The hCG is initially produced by the blastocyst 6–8 days after fertilization ***(Lopata and Hay, 1989).***

It is one of the early embryonic signals in primates that is secreted by the embryo before its implantation ***(Bonduelle et al., 1988).***

Different mechanisms have been described in which hCG can regulate implantation. An in vitro study demonstrated that hCG is a potent attractor of inflammatory cells, such as neutrophils, monocytes, and lymphocytes (***Reinisch et al., 1994).***

The hCG directly regulates endothelial cell responsiveness to interleukin 1 and amplifies the cytokine-mediated effect on cell proliferation, migration and release of angiogenic factors ***(Bourdiec et al., 2013).***

Embryo implantation requires an extensive angiogenesis at the maternal-fetal interface. The hCG can modulate the receptivity of the endometrial stromal cells to interleukin-1 by upregulating its receptor (IL1R) during the implantation window. This function has an impact on angiogenesis, which is a pathway by which embryonic growth is promoted ***(Bourdiec et al., 2012).***

Licht et al. developed an intrauterine microdialysis device to measure paracrine mediators. After the administration of 500 UI of hCG, they found a significant inhibition of intrauterine insulin-like growth factor binding protein 1 (IGFBP-1) and the macrophage colony-stimulating factor (M-CSF), while leukemia inhibitory factor (LIF), the vascular endothelial growth factor (VEGF) and the matrix metallopeptidase 9 (MMP-9) were significantly stimulated. These multiple effects appear to precede the classical endocrine role of the hCG and could be directly involved in the regulation of embryo implantation ***(Licht et al., 2007).***

Mansour et al. reported the first time use of an intrauterine injection of hCG before the embryo transfers, and they found a significant improvement in the pregnancy rates of the IVF cycles (***Mansour et al., 2011).***

Another study was performed by Santibanez et al. which aimed to reproduce and confirm the benefits to the clinical effects of intrauterine injection of hCG before embryo transfer on the pregnancy rates in the IVF cycles ***(Santibanez et al., 2014).***

Aim of the Work

The aim of the present study is to investigate the value of intrauterine injection of hCG at day of ovum pick-up in patients undergoing intracytoplasmic sperm injection in an attempt to improve implantation and pregnancy rates on patients with recurrent implantation failure.

Human Chorionic Gonadotropin

Human chorionic gonadotropin (**hCG**) is a hormone produced by the syncytiotrophoblast, a portion of the placenta following implantation ***(Gregory and Finlay, 1999 and Cole, 2009).***

The presence of hCG is detected in pregnancy tests. Some cancerous tumors produce this hormone; therefore, elevated levels measured when the patient is not pregnant can lead to a cancer diagnosis. However, it is not known whether this production is a contributing cause or an effect of tumorigenesis ***(Cole, 2009).***

The pituitary analog of hCG, known as luteinizing hormone (LH), is produced in the pituitary gland of males and females of all ages ***(Cole 2009, and Hoermann et al., 1990).***

Both hCG and LH bind and function through a common hCG/LH receptor. The biggest difference between LH and hCG is that LH, has a circulating half-life of just 25-30 minutes ***(Parlow et al., 1968)***, while hCG, has a circulating half-life of approximately 37 hours ***(Faiman et al., 1968)***, or 80-fold longer than that of LH.

Structure:

Human chorionic gonadotropin is a glycoprotein composed of 237 amino acids with a molecular mass of 25.7 kDa (***Lapthorn et al., 1994).***

$$\underline{\mathbf{C}}_{1105}\underline{\mathbf{H}}_{1770}\underline{\mathbf{N}}_{318}\underline{\mathbf{O}}_{336}\underline{\mathbf{S}}_{26}$$

Figure (1): Chemical formula of hCG

It is heterodimeric, with an α (alpha) subunit identical to that of luteinizing hormone (LH), follicle stimulating hormone (FSH), thyroid stimulating hormone(TSH), and β (beta) subunit that is unique to hCG.

- The α (alpha) subunit is 92 amino acids long.
- The β-subunit of hCG gonadotropin (**beta-hCG**) contains 145 amino acids, encoded by six highly homologous genes that are arranged in tandem and inverted pairs on chromosome **19q13.3 -*CGB* (*1*, *2*, *3*, *5*, *7*, *8*)**

The two subunits create a small hydrophobic core surrounded by a high surface area-to-volume ratio 2:8 times that of a sphere. The vast majority of the outer amino acids are hydrophilic (***Lapthorn et al., 1994).***

Research shows that there are at least 4 independent variants of hCG, each produced by different cells with separate biological functions. All the molecules share a common hCGβ-subunit amino acid sequence (***Lapthorn et al., 1994).***

There is hCG, produced by differentiated syncytio-trophoblast cells or more specifically villous syncytiotrophoblast