INTRODUCTION

Infertility is defined as the inability to conceive offspring after 12months of unprotected intercourse **(*Zegers-Hochschild et al., 2009*).** Ovulation, patent tubes, normal sperm parameter, and good timing are among the necessary factors for natural pregnancy to occur **(*Smith et al., 2012).*** Worldwide more than 70 million couples afflicted with infertility ***(Ombelet et al., 2008).*** A proportion of these couples may be able ultimately to conceive, but for the majority conception is unlikely without some form of medical intervention ***(Collin, 2004)***.

Implantation rates are still low after ART and controlled ovarian stimulation despite of the great improvements in fertilization procedures. During the implantation period, the endometrium undergoes transition and acquires an appropriate morphological and functional state under the influence of ovarian steroids, which facilitate the attachment of blastocyst. In addition progesterone and estrogen are the dominant hormonal modulators of endometrial development. Progesterone is essential for implantation and pregnancy maintenance in all mammals, whereas the requirement for estrogen is species specific ***(Dadi et al., 2009).***

Implantation failure is thought to result from impairment of embryo development and/ or from abnormal uterine receptivity ***(Delphine et al., 2012*).**

The favorable influence of local injury of the endometrium was confirmed by ***Raziel et al. (2007)*** and ***Karimzadeh et al. (2009)*** The association of mechanical manipulation with decidual formation has been reported previously in rodents (***Lejeune et al., 1982)***, these early observed in rodents, combined with others in humans suggest that local injury of the endometrium facilitates successful implantation. (***Lejeune et al., 1982).***

The success rate of IUI with ovulation induction varies widely, with pregnancy rates ranging between 8 and 18% per cycle ***(Goverde, 2000).*** Implantation failure and its associated defects remain as unresolved problem in reproductive medicine and are considered a major cause of infertility in otherwise healthy women ***(Achache et al., 2006).***

Implantation of the embryo, which is a prerequisite for successful pregnancy, can only take place in a receptive uterus. In humans, the uterus becomes receptive during the mid-secretory phase of the menstrual cycle (days 19–23), commonly known as the window of implantation (WOI). It is assumed that inadequate uterine receptivity is responsible for approximately two-thirds of implantation failures ***(Gnaisky et al., 2010)***.

Endometrial injury triggers a series of biological responses but the findings to date suggest that no particular

pathway is solely adequate to explain the association between trauma and improved pregnancy rates rather than a cluster of events in response to trauma which benefits embryo implantation in ways both known and unknown to the scientific community ***(Siristatidis et al., 2014).***

The current study is evaluating the hypothesis that, the endometrial injury procedure may or may not improve pregnancy rates in patients undergoing controlled ovarian hyperstimulation (COH) combined with intrauterine insemination (IUI), with endometrial injury performed in the same cycle of IUI versus control group undergoing IUI without endometrial injury.

AIM OF THE STUDY

To evaluate the effect of endometrial injury in improving the pregnancy rates in controlled ovarian hyperstimulation and intrauterine insemination cycles.

Chapter (1)

ENDOMETRIAL RECEPTIVITY

Uterine receptivity is defined as a limited period when the uterine environment is conducive to blastocyst acceptance and implantation. ***(Zhaowei et al., 2014).***

Implantation

Several factors are involved in the fetal maternal interactions, including hormones, growth factors, cytokines, chemokines, adhesion molecules, extracellular matrix components, matrix degrading enzymes ***(Garlanda et al., 2008).***

Implantation Window

Endometrium is known to become receptive only for short periods. Beyond this period of receptivity, the embryo is unable to successfully establish contact with refractive endometrium. Therefore, timely arrival of embryo in a receptive endometrium is very much crucial for successful implantation.This time period is called the 'window of implantation', during which the uterine environment is conducive to blastocyst implantation. ***(Ma et al., 2003).***

Essential expression of proteins, cytokines, and peptides can be detected at this time and serve as biomarkers for maximal endometrial receptivity ***(Singh et al., 2009).***The establishment of the window of implantation is under the control of steroid hormones, which perform their effects through local mediators ***(Kimber, 2005).***

Implantation Failure

Implantation failure has diverse causes, including abnormal cytokine and hormonal signaling as well as epigastric alterations. Recurrent implantation failure is a cause of female infertility. Therefore pregnancy rates can be improved by optimizing endometrial receptivity for implantation. Evaluation of implantation markers may help to predict pregnancy outcome and detect occult implantation deficiency ***(Cakmak et al., 2010).***

Role of Hormones

The ovarian steroids, progesterone and estrogen, have major regulatory role by mobilizing several molecular modulators in a spatiotemporal manner, which supports embryo implantation ***(Lim et al., 2002).*** During the implantation period, the endometrium undergoes transition and acquires an appropriate morphological and functional state under the influence of ovarian steroids, which facilitate the attachment of blastocyst. In addition progesterone and estrogen are the

dominant hormonal modulators of endometrial development. Progesterone is essential for implantation and pregnancy maintenance in all mammals, whereas the requirement for estrogen is species specific ***(Dey et al., 2004).***

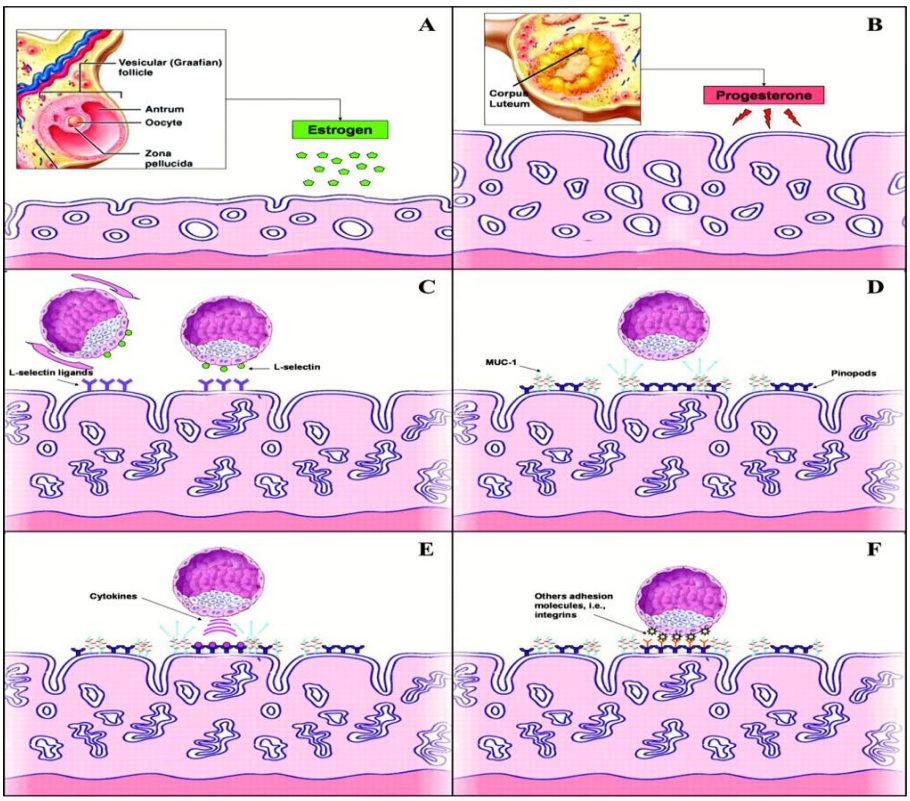

Fig. (1): Human embryo implantation in the uterus. (A) Endometrium proliferates under estrogen enhancement. (B) Progesterone from luteinized follicles leads to endometrial differentiation. (C) The blastocyst enters the uterus through the ostia and rolls freely over the endometrium under signals by L-selectin. (D) Mucin-1 (MUC-1) repels the blastocyst and prevents its adhesion to endometrial areas with poor chances of implantation. (E) Chemokines and cytokines attract the blastocyst to the optimal implantation spot. (F) Adhesion molecules (e.g. integrins and cadherins) firmly attach the blastocyst to the endometrial pinopods to ensure further successful implantation ***(Achache and Revel 2006).***

Biomarkers of endometrial receptivity

Pinopodes

Pinopodes are ultrastructural projections on the apical surface of the luminal epithelium, which appear only during the receptive phase. They have been detected by electron microscopy and are specific markers for uterine receptivity. Progesterone dependent, pinopodes are present 20-21 days into luteal cycle ***(Cavagna et al., 2003).***

HLA-G

At first, HLA-G was proposed as a protector against natural killer (NK)-cell-mediated cytolysis of target cells and to prevent all recognition by maternal cytotoxic lymphocytes. Recently, it has been shown that these proteins regulate immune cells including T cells, NK cells, and antigen-presenting cells ***(Fournel, 2000).***

Due to its essential role in the implantation process, recent attention has been focused on HLA-G and its diagnostic and therapeutic clinical applications. This has included the evaluation of couples with recurrent miscarriages and the mutation of the HLA-G gene. Serum sHLA-G levels during pregnancy may in the future become a diagnostic tool for evaluation of successful implantation but has yet to be established ***(Roussev et al., 2007)***.

Integrins

The integrin family of cell adhesion molecules is a major class of receptors for the extracellular matrix protein (ECM) and participates in cell-cell and cell-substratum interactions. They have many functions in cellular processes including differentiation, apoptosis and cell survival, motility and attachment ***(Desgrosellier et al., 2010).***

Integrins regulates the adhesions between cells and extracellular components ***(Ceydeli et al., 2006).*** Patients with normal integrin levels had a double pregnancy rate as compared with patients with low levels. Implementation of integrin ß3 expression may thus be a useful tool to predict success in an IVF program ***(Revel et al., 2005)***.

Selectins

Hatched blastocysts expressed L-selectin and used this molecule to mediate its attachment to the luminal epithelial surface, its carbohydrate ligands, and related epitopes ***(Foulk et al., 2007)***. Elevated L-selectin ligand has been associated with improved implantation (***Wang et al., 2008).***

Cadherins

During the luteal phase, E-cadherin mRNA levels are significantly elevated and regulation seems to be mainly

controlled by intracellular calcium levels. E-cadherin cytoskeletal organization and disassembly at the adherens junction are mediated by rising levels of calcium which work by acting on signaling pathways. In vitro studies have shown that calcitonin produces a transient rise in intracellular calcium levels, suppressing E-cadherin at cellular contact sites ***(Mark et al., 2012)***

Endometrial anti-adhesion molecules (Mucins)

Mucins are glycoprotein family existing in the surfaces of human epithelial cells. In human, they are present at high levels during the peri-implantation period. Among the mucins which form anti-adhesive molecules in this layer, MUC1 is the most important ***(Bahar et al., 2008).***

Cytokines

Cytokines are small multifunctional glycoproteins, whose biological actions are mediated by specific cell surface receptors and act as potent intercellular signals regulating functions of endometrial cells and embryo–maternal interactions. Entry of blastocyst into the receptive uterine is very important for the production of cytokines by trophoblastic cells and uterine epithelium that can modulate the endometrial receptivity by regulating the expression of various adhesion molecules ***(Simon et al., 2000).***

Deregulated expression of cytokines and their signaling leads to an absolute or partial failure of implantation and abnormal placental formation (***Guzeloglu-Kayisli et al., 2009).***

Leukemia Inhibitory Factor (LIF)

Infertile patients and those with repeated implantation failures have been shown to have abnormal levels of LIF supporting the role of LIF as a fundamental element in the implantation process. ***(Achache et al., 2006)***

It has been shown that LIF plays a role in both adhesive and invasive phases of implantation due its anchoring effect on the trophoblast (***Dimitriadis et al., 2010).***

Interleukin 6 (IL-6)

Fewer reports are available regarding the role of IL6 in pregnancy ***(Mohan et al., 2011).*** mRNA expression of IL-6 steadily increases during the mid- to late-secretory phase and then decreases again in late-secretory phase. During the crucial window of implantation, immunoreactivity for IL-6 becomes markedly detectable ***(Achache et al., 2006).***

IL-6 is also involved in decidulization tissue remodeling and the development of placenta ***(Singh et al., 2011).***

Interleukin 11 (IL-11)

IL-11 is a multifunctional cytokine with anti-inflammatory properties ***(Dimitriadis et al., 2005).*** The most prominent immunoreactivity and mRNA expression is in the decidualized stromal cells late in the menstrual cycle ***(von Rango et al. 2004)*** despite this, there is still uncertainty regarding the time of maximal production of IL11 in the epithelial cells***. (Mohan et al., 2011)***

Thus, all variation in level of activity depends on IL-11 production, which is influenced by steroid hormones and by more local factors such as relaxin and PGE_2. A defect in IL-11 or its receptor may be involved in certain cases of human infertility. However, the significance of these findings remains to be determined ***(Dimitriadis et al., 2005)***.

Interleukin 1 (IL-1)

IL-1 has several functions in the window of implantation. It stimulates the production of LIF by the endometrium ***(Kimber et al., 2005)*** and the production of leptin and its receptor ***(Dimitriadis et al., 2005).***

In women with endometriosis, the levels of interleukin-1 receptor antagonist (IL-1ra) and interleukin-1 alpha (IL-1α) were found to be markedly increased when compared to control

groups in the peritoneal fluid and serum and may serve as an explanation of the pathogenesis and infertility in such patients. ***(Kondera-Anasz et al. 2005)***

Leptin

Acting both at the endocrine and paracrine level, leptin has been associated with regulation of body weight and reproductive function ***(Cervero et al., 2004)***. Additionally, leptin has also been shown to increase integrin β3 expression, an important ligand protein essential for endometrial receptivity and implantation. The leptin receptors OB-RT gene, OB-RL, HuB219.1, and HuB219.3 have all demonstrated maximal expression in the late luteal phase ***(Achache et al., 2006).***

Prostaglandins

It was documented that prostaglandins (PGs) play an important role in various reproductive processes, including ovulation, implantation, and menstruation ***(Kang et al., 2005).***

Extensive research in past years provides crucial evidence confirming the role of PGs in implantation process. Patients with recurrent pregnancy failure were shown to have very low levels of cPLA2α and COX-2, possibly reducing further PG synthesis, which could be responsible for implantation failure ***(Achache et al., 2010).***

Cyclin E

Cyclins are known to control mitotic phase progression in cells. The G1 to S phase transition is controlled by the rate limiting step of Cyclin E, whereas prevention of the cell cycle progression is controlled by the p27 cyclin-dependent kinase inhibitor. ***(Kliman et al., 2006)*** While the plausible role of cyclin E involves proliferation, p27 is mostly responsible for differentiation**.** ***(Dubowy et al., 2003)*** Consistent with these actions, estrogen has positive regulatory effects on Cyclin E and progesterone seems to induce a dominant p27 state. Cyclin E activity is present in the cytoplasm of epithelial cells whereas p27 activity is exclusively active in the nucleus. While present in the early phases of the menstrual cycle, Cyclin E reactivity seems to rapidly decrease after cycle day 19; this could be explained by its subsequent movement towards the nucleus where it binds to p27 thereby becoming inactivated**.** ***(Mark et al., 2012).***

Chorionic gonadotropin and Notch 1

Chorionic gonadotropin is one of the early embryonic secretions from the trophoblast cell of the pre-implantation embryo. This helps maintain the corpus luteum of pregnancy and leads to the modifications in morphology and endometrial gene expression preparing for implantation ***(Afshar et al., 2007)***.

Notch proteins are ligand-dependant transmembrane receptors that transduce extracellular signals responsible for cell-fate and differentiation throughout development. Evidence indicates that notch signaling regulates all three branches of the fate cell decision tree; differentiation, cell cycle progression and apoptotic cell death ***(Afshar et al., 2007)***.

Colony Stimulating Factor-1 (CSF-1)

CSF-1 is a haemopoietic growth factor inducing proliferation and differentiation of cells belonging to the mononuclear phagocytic lineage. It has been shown that women with lower pre-conceptional CSF-1 levels are more prone to recurrent abortions compared to women with higher pre-conceptional CSF-1 levels ***(Cavagna et al., 2003).***

Nuclear pore complex proteins

Nuclear channel systems (NCSs) are membranous organelles appearing transiently in the epithelial cell nuclei of postovulatory human endometrium. The immunodetection assay combined with the hitherto underappreciated prevalence of NCSs now enables simple screening and further molecular and functional dissection ***(Guffanti et al., 2008).***

Serum-and Glucocorticoid-Regulated Kinase 1 (SGK 1)

Recently, ***Feroze-Zaidi el al., (2007)*** demonstrated that women with unexplained infertility or recurrent implantation