Role of measuring the endometrial thickness and assessment of sub - endometrial vascularity by power Doppler ultrasound in prediction of pregnancy rate in patients undergoing IVF/ICSI cycle

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ABSTRACT

Objectives**:* evaluation of the role of the measurement of endometrial thickness and assessment of subendometrial blood flow by 2D Power Doppler ultrasound performed on the day of hCG triggering in patients undergoing IVF/ICSI cycle in prediction of pregnancy rate. ***Methods**:* fifty infertile patients candidate for IVF/ICSI treatment received down regulation with GnRH-agonists followed by ovarian hyperstimulation with HMG, on day of HCG triggering, Endometrial thickness was measured, and Resistance index (RI), Pulsatility index (PI), (S/D) ratio were assessed by Power Doppler TVU. ***Results: mean endometrial thickness was found to be statistically insignificant (P value for pregnancy group: **0.433**), and so are the RI, PI, and S/D ratio, P values were (**0.256, 0.173, 0.186**) respectively. ***Conclusion:*** measurement of endometrial thickness and assessment of subendometrial blood flow by 2D Power Doppler ultrasound performed on the day of hCG triggering in patients undergoing IVF/ICSI cycle cannot predict pregnancy rate.

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RI: Resistance index
PI: Pulsatility index
S/D ratio: Systolic/diastolic ratio
IVF: In vitro Fertilization
ICSI: Intra cytoplasmic sperm injection
COH: controlled ovarian hyperstimulation
TVU: trans vaginal ultrasound
HMG: human menopausal gonadotropine
GnRH: gonadotropin releasing hormone
IL: interleukin
LIF: leukocyte inhibitory factor

Introduction

Introduction

Despite recent advances in IVF technologies and ovarian stimulation regimens, the pregnancy rates have not increased accordingly, Multiple factors responsible for a successful IVF outcome have been described, not the least of which is uterine receptivity **(Coulam *et al*, 1994).**

It appears that a favorable endometrial milieu is necessary for successful implantation in the menstrual cycle, the endometrium has no adhesive qualities until the implantation window phase, during which for a very short time, the endometrium allows the implantation of gestational sacs. This feature is referred to as endometrial receptivity **(Dominguez *et al*, 2003).**

With the advance of diagnostic ultrasonography, clinical use of ultrasonic technology has increased as a way to measure possible predictors of endometrial receptivity, among them are uterine predictors of implantation, such as endometrial thickness, in the assessment of the developmental potential of the basal layer of the endometrium. With the increased resolving power and sensitivity of ultrasonography, more studies were conducted on the use of endometrial blood flow and blood flow in the (sub) endometrial arteries in predicting endometrial receptivity **(Schild *et al*, 2000).**

To date, the advantages of ultrasonography include its non-invasiveness, repeatability, real-time monitoring and predictability **(Wang *et al*, 2010).**

Endometrial thickness measurement is used as a clinical tool to predict implantation following ovarian stimulation for IVF.

Endometrial thickness is defined as the minimal distance between the echogenic interfaces of the myometrium and endometrium measured in the plane through the central longitudinal axis of the uterine body.

There is possible interaction between overall blood supply in the sub endometrial area and pregnancy rate **(Yu Ng *et al*, 2006).**

Using a transvaginal transducer with power Doppler facility, when a longitudinal view of the uterus is obtained, sub-endometrial blood flow can be studied measuring the following parameters:

i. Resistance index (RI) unitless and angle independent: the difference between maximal systolic blood flow and minimal diastolic flow divided by the peak systolic flow (S-D/S).
ii. Pulsatility index (PI) unitless and angle independent: the difference between maximal systolic blood flow and minimal diastolic flow divided by the mean flow throughout the cycle (S – D/ mean).
iii. The ratio between peak systolic flow and lowest diastolic flow (S/D).

These three parameters express the resistance to flow from the point of measurement downstream. The value increases when resistance increases, and vice versa. The diastolic flow is considered to be influenced by resistance to a greater extent than the systolic flow. All parameters are also examined by using the power Doppler system.

Aim of the work

Aim of the work:

Is to study the correlation between (endometrial thickness, and assessment of sub-endometrial vascularity, by power Doppler ultrasonography), and uterine receptivity in infertile women treated with IVF/ICSI.

Assessment of endometrium during induction cycle

The endometrium is a dynamic organ , composed of multiple layers, overlying the myometrium , two distinct layers are observed within the endometrium, the basal layer which remains adherent to the myometrium and the functional layer which in absence of pregnancy is shed with each menstrual cycle, many cell types are identified within the endometrium, glandular, luminal cells, stromal, stromal fibroblastic cells, and immune cells, the composition of these cells change throughout the menstrual cycle and pregnancy.

Endometrium is hormonally regulated; it is highly specialized in that it does not become "receptive", thus allowing implantation except for a very short time in menstrual cycle 'implantation window' or "window of uterine receptivity", which takes place on day 19 to 24 of the cycle, the exact mechanism by which the endometrium undergoes the transition between non- receptive to receptive is poorly understood, however, during the follicular phase, Estradiol stimulates endometrial proliferation, which is followed by endometrial differentiation in the luteal phase under the effect of progesterone, certain structural and functional modifications are observed during the period of implantation.

Historically, endometrial receptivity was assessed morphological characteristics, and by using single markers, advances in US technologies permits the use of DNA microchips and proteomic analysis in monitoring small changes at the level of thousands of genes or proteins respectively **(*K. Diedrich et al., 2007)***.

Histological analysis of the endometrium

***Noyes et al., (1950); (Noyes and Haman, 1953*)** examined the endometrium during various points of spontaneous menstrual cycles, by taking endometrial biopsies, and concluded criteria for endometrial dating in which they linked

specific histological patterns to specific points of time in menstrual cycles, this method of endometrial dating was the gold standard to detect endometrial responsiveness and to diagnose endometrial abnormalities.

According to **(*Noyes et al., 1950)***, certain features were noted: 1. Gland mitosis, 2. Pseudo-stratification of nuclei, 3. Basal vacuolation, 4. Secretion, 5. Stromal edema 6. Pseudo-decidual reaction, 7. Stromal mitosis, 8. Leukocytic infiltration. Advantages of histological assessment of uterine receptivity by Noyes is that it enables assessment of both morphology and function of the cells, and differential component analysis, while weak points are related to the fact that it disregards the embryo component and contribution to the process of implantation, only provides information on the endometrium at time of biopsy, subjective interpretation of the sample and sample bias because the sample taken may not reflect the whole state of endometrium as it is not applicable to take large number of samples **(*Diedrich et al., 2007)***, moreover, endometrium biopsy at the time of the window of implantation could have proved hazardous for embryo implantation ***(Labarta et al, 2011)***, however, Endometrial dating is informative during the follicular, early and late luteal phase, as mid luteal phase (window of implantation) lack specific morphological characteristics: stromal edema is the only feature ***(Diedrich et al., 2007)***, moreover, it does not discriminate between fertile and infertile women and is therefore not a valid tool in routine evaluation of infertility or implantation failure ***(Cakmak and Taylor, 2011; Coutifaris et al.2004)***.

Molecular method of endometrium assessment

Electron microscopy is used to study the endometrial cells ultra-structures during the implantation window, like nucleolar channels and pinopodes, Pinopodes are apical cellular protrusions that become visible between Days 20 and 21 of the natural menstrual cycle ***(Cakmak and Taylor, 2011; Nikas and Aghajanova, 2002)***, may be prove useful markers for endometrial receptivity ***(Diedrich et al; Bentin-Ley et al, 1999, Isaac C et al, 2001)***, human blastocyst attaches

preferentially to pinopodes presenting areas **(*Diedrich et al., 2007; Bentin-Ley et al, 1999*)**.

Advances in biotechnology has allowed for examination of gene expression and protein production throughout the menstrual cycle, using DNA array technology to detect gene expression is a more reliable predictor of uterine receptivity than morphological characteristics of endometrial cells ***(Diedrich et al., 2007)***, evidence suggests that the expression of molecular genes change over the course of menstrual cycle ***(Diedrich et al., 2007; Ponnampalam et al. 2004; Talbi et al.2006)***, one gene (osteopontin) was shown to be extensively up-regulated and expressed during window of implantation***(Diedrich et al., 2007; Carson et al.2002; Kao et al.2002; Borthwick et al.2003; Riesewijk et al.2003; Mirkin et al.2005)***.

Molecular markers of endometrial receptivity include: Growth factors & Cytokines (LIF, IL-1, and IL-6), Lipids (prostaglandins), Cell adhesion molecules (integrins, L-selectin and Cadherins).

Advantages of measuring changes in protein or gene expression to assess the endometrial receptivity is being an objective approach, provide information in a short time about large group of related molecules.

Methods were also invented in order to examine the protein production of endometrial cells during menstrual cycle, proteomic analysis, using endometrium aspiration, the advantage of this method is that it has no adverse effect on embryo and implantation ***(Diedrich et al., 2007; van der Gaast et al, 2003.)*** , but specimens aspirated are subject to a great deal of cellular contaminations.

Dubowy et al, (2003.) developed an Endometrial Function Test (EFT) that involves 'immunohistochemically staining of the endometrium with markers for the mitotic regulators cyclin E (the rate-limiting activator of the mitotic G1–S phase transition) and p27 (an inhibitor of cyclin E)' (***Cakmak and Taylor, 2011 ;Dubowy et al, 2003.)***, the use of endometrial receptivity markers have not been adopted