Endometrial Blood Flow Predicting Receptivity of Endometrium in Assisted Reproductive Technology

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List of Abbreviations

2-D	Tow-dimensional
3-D	Three-dimensonal
APA	Antiphospholipid antibodies
ART	Assisted reproductive technology
BMI	Body Mass Index
CAM	Cellular adhesion molecules
E2	Estradiol
ECM	Extracellular membrane
EECs	Endometrial epithelial cells
ER	Estrogen receptor
FSH	Follicular stimulating hormone
GnRH	Gonadotrophin-releasing hormone
HB-EGF	Heparin Binding epidermal growth factor
hCG	Human chorionic gonadotrophin
HMG	Human menopausal gonadotrophins
HOX	Homeobox
ICSI	Intracytoplasmic sperm injection
IGFBP	Insulin like growth factor binding protein
INF	Interferon

IVF	In-vitro fertilization
LH	Lutinizing hormone
LIF	Leukaemia inhibitory factor
MHZ	Mega Hertiz
MUC	Mucin
PCOS	Polycystic ovarian syndrome
PCR	Polymerase chain reaction
PI	Pulsatility index
PR	Progesterone receptors
PR-B	Progesterone receptor-B
RCT	Randomized controlled trial
RI	Resistance index
S/D	Systolic / diastolic ratio
SD	Standard deviation
TGF	Transforming growth factor
TNF	Tumor necrotic factor
VNTR	Variable number of tandem repetition

INTRODUCTION

Since 1978 when the first in-vitro fertilization (IVF) baby was born, the processes involved have gradually improved and are currently simplified to the stage of routine clinical and laboratory protocols. It is now very easy to achieve in average of 15 oocytes and up to 70% egg fertilization rate during IVF, yet the average live birth rate remains under 30%. The cellular and molecular interactions between the endometrium and implanting blastocyst remain poorly understood. Implantation failure is therefore an important rate-limiting step during IVF. The definition of implantation failure varies from place to place. The most frequently used criteria, however, are repeated, consecutive failure of embryos to implant following three attempts at IVF ***(Ola and Li., 2006)***, despite good hormonal reserve (FSH of <8) after transfers of fresh embryo with at least two embryos of good quality ***(Matteo et al., 2007)***.

The success of assisted reproductive technology depends upon the intricate relationship between the transferred embryo and the endometrium. Failure of embryo to implant may be because of several factors, most of which have not been delineated ***(Feng et al., 2008)***.

Opinions on the causes of repeated implantation failure are no less controversial. For example, some consider the diagnoses of implantation failure only if good quality embryos

has been transferred unsuccessfully on at least three occasions; therefore, implying that women with poor embryos were not eligible for referral.However, an increasing number of gynecologists consider that inability to produce good quality embryos is itself an important predisposing factor to repeated implantation failure ***(Ola and Li, 2006)***.

The major obstacle to reproductive success is our inability to diagnose or treat the non receptive endometrium ***(Makker and Singh, 2006)***.

Implantation requires synchronization between the developing embryo and endometrium. The dialogue between embryo and endometrium and the receptivity of the latter is under the control of the sex steroids, estrogen and progesterone as well as other hormones ***(Kodaman and Taylor, 2004)***.

In the setting of IVF, implantation efficiency is dependent on three factors: embryo quality, endometrial receptivity and embryo transfer skills ***(Leach, 2001)***.

Uterine Receptivity

Endometrial receptivity is a key determinant of the success of implantation ***(Matteo et al., 2007)***.

Uterine receptivity and embryo implantation are critical in the establishment of pregnancy. The diagnosis of endometrial fertility requires more precise measurements of endometrial receptivity ***(Dimitriadis et al., 2007)***.

This is commonly referred to as the 'implantation or nidation window'. Various architectural, cellular, biochemical, and molecular events in the endometrium are coordinated within the 'implantation window' and constitute essential elements in the repertoire that signifies endometrial receptivity. The window of implantation in humans span cycle days 20-24 (i.e., 5-9 days post-ovulation) during the mid-secretory or the mid-luteal phase ***(Wilcox et al., 1999)***.

Studies based on pattern of hCG in natural pregnancies, too have suggested that the window of implantation might extend up to day 10 after ovulation ***(Lenton et al., 1982)***. Similar range in the window of implantation among women can be postulated from studies in donor oocyte recipients with successful pregnancies occurring after transfer of day 2 and day 3 embryos on days 15-20 of menstrual cycle ***(Novot et al., 1991)***.

Some women may be subfertile because of an unusually short window of implantation ***(Wilcox et al., 1999)***. There is accumulating evidence that the endometrium in humans may even be more receptive to implantation in women with recurrent miscarriage than in normal controls ***(Quenby et al., 2002)***.

The loss of communication or synchrony between the embryo and the endometrium occurs in both animals and humans ***(Lessey and Castelbaum, 2002)***, and is likely a major cause of infertility and pregnancy loss ***(Donoghay and Lessey, 2007)***.

Cellular/ molecular markers and mechanisms underlying implantation

Numerous studies have investigated potential markers of endometrial receptivity as predictors of successful implantation and, in doing so, have helped to define the cellular and molecular mechanisms by which implantation occurs. These markers include pinopodes, cell adhesion molecules, cytokines, homeobox (HOX) genes, growth factors, matrix metallo-proteinases, and their inhibitors. Many clinical situations in which implantation is impaired (eg, hydrosalpinx) are associated with normal estrogen and progesterone levels ***(Kodamon and Taylor, 2004)***.

Pinopodes

With the onset of the secretory phase of the menstrual cycle, microvilli on the apical surface of the luminal endometrial epithelium fuse to form structures that are known as pinopodes ***(Nikas, 2000)***. The appearance of pinopodes coincides with increased progesterone levels and the down-regulation of PR-B during the window of implantation ***(Stavreus-Evers et al., 2001)***. The volume of uterine fluid is decreased during the window of implantation; this phenomenon is not seen following treatment with RU486, an antiprogestin ***(Genzell-Danielsson et al., 1994)***.

Pinopodes last for only 1 or 2 days – usually Days 20 and 21 in an ideal cycle-although there is up to 5 days of variation in the timing of their appearance ***(Nikas, 2000)***. Furthermore, their numbers correlate with implantation ***(Nikas, 1999)***. Pinopodes form earlier in gonadotropin-stimulated cycles (Days 19-20) ***(Nikas et al., 1999)*** and later in artificial, hormone replacement cycles for donor recipients (Days 21-22) ***(Develioglu et al., 1999)***.

Endometrial pinopodes development is associated with the midluteal phase increased expression of leukaemia inhibitory factor (LIF) and its receptor ***(Aghajanova et al., 2003)***. Progesterone ***(Staureus-Evers et al., 2001)*** and integrin αVB3 ***(Lessey et al., 1992)***.

Cellular adhesion molecules family

The cell adhesion molecule (CAM) family is composed of four members known as integrins, cadherins, selectins and immunoglobulins. These surface ligands, usually glycoproteins, mediate cell-to-cell adhesion. Their classical functions include maintenance of tissue integration, wound healing, morphogenic movements, cellular migrations and tumour metastasis ***(Achache and Revel, 2006)***.

i. Integrins

Perhaps the best studied of the CAMs have been the integrins ***(Lessey and Castlebaum, 2000)***. Integrins are a family of transmembrane glycoproteins, formed by the association of two different, α and β subunits. To date, 18 α and eight β chains have been identified in mammals ***(Hynes, 2002)***. These subunits contain extracellular, transmembranal and intracellular domains. The extracellular domain enables integrins to act as a receptor to ECM components [fibronectin (FN), laminin and collagen type IV], complement and other cells ***(Gilmore and Burridge, 1996)***.

Integrins whose expression is increased in mid-luteal phase were proposed as markers for the frame of the window of implantation ***(Lessey et al., 2000)***. Three cycle-specific integrins are co-expressed by the human endometrium defined