Introduction

Infertility is a common problem affecting approximately 13-14% in couple's reproductive age. The classic definition of infertility is the inability to conceive after 1 year of unprotected intercourse *(**Abma et al., 1997**)*.

Studies of populations of patients with infertility indicates that approximately 10-25% have unexplained infertility,20-30% ovulatory dysfunction, 20-25% tubal damage, 10-50% sperm dysfunction, 5-10% endometriosis, 5% cervical mucous problems and 5% coital dysfunction while a large number of human pregnancies fail during implantation stage because the uterine endometrium is unreceptive to the implanting embryo ***(Jacobs and Balen, 2003)***.

The past 50 years have been a revolution in the regulation of the female reproductive system with the development f steroid and now gonadotrophin therapies. These advances have led to hormonal contraception, hormone replacement therapy, ovulation induction and have facilitated the growth of assisted reproduction ***(Smith and Brien, 1998).***

Recent development in infertility treatment have further diminished the consequences of sever male factor infertility.In-Vitro Fertilization (IVF) presented a new hope to many couples

who would otherwise have absent or minimal chance of conception.It is a fertility procedure which first succeeded as recently as 1978 by Dr. Edward (an embryologist) and Dr. teptoe (agynecologist) in England embryologist ***(Steptoe and Edwards, 1979)***.

IVF/embryo transfer (IVF/ET) treatment involves development of multiple follicles, oocyte retrieval and ET after fertilization.Since then the technology has been further refined and developed by physician and embryologist. Intracytoplasmic sperm injection (ICSI) is one of the modalities of assisted reproductive technique which aims to the treatment of couples in whom the male partners has azoospermia or sever oligospermi. ICSI is also indicated for men with significant antisperm antibodies, low sperm motility, or significantly abnormal sperm morphology. ICSI is used when poor fertilization occurs with regular insemination techniques in the laboratory. Sperm can be obtained from the ejaculate or directly from the pididymis. Recently, success was obtained with spermatids from testicular biopsies ***(Palermo et al., 1992).***

Failure of conception despite the repeated transfer of apparently good quality embryo is a significant clinical problem in vitro fertilization (IVF) practice. Clinically, the definition of recurrent treatment failure varies but usually includes a number of completed IVF-embryo transfer (ET) cycles and/or the

cumulative number of embryos transferred during the unsuccessful treatment ***(Stern et al., 2003)*** ***(Raziel et al., 2002)***.

Success rate in IVF remain stubbornly low, providing distress both for the individual concerned and for the economics of the women ´s health. There is considerable interest in the potential causes of recurrent implantation failure and in strategies that may improve implantation through changes in clinical and embryology practice ***(Scott, 2002)***.

IVF success is dependent on the coordinated development of embryo and endometrium and persistent abnormalities of either may lead to recurrent treatment failure ***(Ng et al., 2006).***

Because of the major medical, emotional and financial implication of IVF treatment, numerous studies addressed different methods of trying to predict the outcome of IVF cycles, and to define possible risk factors that may underlie any poor outcome, in a hope to improve the success rates of IVF. Some of these include immunological testing and treatment ***(Urman, 2005 a).***

Increased levels of autoantibody were initially described in patients with infertility due to endometriosis, but recurrent spontaneous abortion (RSA) and infertility patients overall have higher levels of autoantibody than do fertile woman ***(Gleicher et al., 1989).***

Autoantibody included in infertility screening often include ANA, APL, antithyroid antibodies, antisperm antibodies, and antizonal antibodies ***(Kutteh, 2002).***

An increased prevalence of APL in patients with infertility has been shown in a large number of studies. Prevalence of APL in normal, including healthy pregnant women, is about 2% to 5%; in patients with RSA, prevalence is 15% to 20% ***(Branch and Khamashta, 2003)***.

The specific APL measured in these studies range from the generally accepted ACL and LA to large panels of multiple autoantibodies directed against various phospholipids. Some authors, however, feel that certain APLs distinct from ACL and LA are more predictive of IVF failure ***(Balash and Cervera, 2002)***.

Studies including only IVF failure patients show roughly the same range of prevalence rates as general infertility patients, from 10% to 32%. However, direct comparison studies do suggest a higher prevalence in patients with implantation failure ***(Chilcott et al., 2000).***

The endometrium is likely to be a key determinant in successful implantation but its individual contribution relative to that of the developing embryo is an area of continued debate ***(Schwartz et al., 1997).***

This partly reflects the current assessment of the endometrium, which is invariably restricted to a simplistic measurement of its thickness and description of its appearance ***(Turnbull et al., 1995).***

These parameters are important in that conception is less likely to occur in patients with thinner endometria or in those with aberrant echogenic patterns, but they are not specific and their value as prognostic indicators of implantation following embryo transfer is limited ***(Friedler et al., 1996).***

Whilst there are several clinically useful tests available that may uncover ovarian dysfunction, the endometrial requirements for implantation remain an enigma. Angiogenesis plays a critical role in various female reproductive processes such as development of a dominant follicle, formation of a corpus luteum, growth of endometrium and implantation ***(Abulafia and Sherer, 2000; Smith, 2001).***

Endometrial receptivity can be evaluated by histological examination of an endometrial biopsy, endometrial proteins in uterine flushing ***(Li et al.,1998)***or more commonly noninvasive ultrasound examination of the endometrium ***(Friedler et al., 1996).***

Doppler ultrasound assessment of the uterine blood supply appears to be informative. Pulsed wave Doppler waveform

indices of high resistance to flow within the uterine artery have been repeatedly linked to poor outcomes during assisted reproduction treatments *(**Sterzik et al., 1989; Steer et al., 1992; Coulam et al., 1994; Levi-Setti et al., 1995**).*

Controlled ovarian stimulation itself, however, has a significant effect on uterine perfusion ***(Kupesic and Kurjak, 1993),*** making information derived from patients undergoing assisted reproduction not necessarily representative of women with unexplained subfertility as a whole.Surprisingly there is a relative paucity of work specifically looking at pelvic blood flow in these women during a spontaneous menstrual cycle.

Aim of the Work

To assess endometrial thickness, endometrial pattern and subendometrial blood flow using colour Doppler ultrasound for prediction of endometrial receptivity in ICSI.

Basics of Implantation

Introduction:

Successful embryo implantation is a crucial event in natural and assisted human reproduction. Blastocyst implantation is a dynamic process, involving embryo apposition, attachment to the maternal endometrial epithelium, and invasion into the endometrial stroma ***(Hanna and Ariel, 2006)***.With in vitro fertilization (IVF), implantation failure can occur due to several Factors ***(Levi et al., 2004)***, including poor embryo quality which is identified as a major cause of implantation failure ***(Urman et al., 2005) .***

Another widely acknowledged barrier to successful blastocyst implantation is an inappropriately developed endometrium. It is well established that embryos cannot implant in a poorly matured endometrium ***(Hanna and Ariel, 2006)***and this may be responsible for low implantation rates with transfer of "good quality" embryos. Moreover the success of embryonic implantation further relies upon cross talk between the embryo and a receptive endometrium ***(Hanna and Ariel, 2006).***

Even though the blastocyst can implant in different human tissues, surprisingly in the endometrium, this phenomenon can only occur during a self-limited period (implantation window), it is understood to be the period of maximal uterine receptivity,

and is believed to commence 7 days after ovulation (around Day 20 of an idealized 28-day cycle) and lasts no more than 2 days ***(Cavagna and Mantese, 2003)***, the human endometrium is primed for blastocyst attachment, given that it has acquired an accurate morphological and functional state initiated by ovarian steroid hormones ***(Paria et al., 2002).***

Implantation failure remains an unsolved problem in reproductive medicine and is considered as a major cause of infertility in otherwise healthy women. Indeed, the average implantation rate in IVF is around 25% ***(de los Santos et al., 2003).*** Inadequate uterine receptivity is responsible for approximately two-thirds of implantation failures, whereas the embryo itself is responsible for only one-third of these failures ***(Ledee-Bataille et al., 2002; Simon et al., 1998).***

A-Endometrial role in implantation:

I) Endometrial morphological features:

1-Histology:

The endometrium is a multilayered, dynamic organ overlaying the myometrium and comprises a functional layer and a basal layer. Each month, cells in the functional layer are separated from the basal layer during menstruation. The basal layer is attached to the myometrium and remains intact during menstruation, serving as a base for endometrial regeneration.

The endometrium is composed of several different cell types, including luminal and glandular epithelial cells, stroma with stromal fibroblastic cells, immunocompetent cells and blood vessels. The numbers, activity, structure and function of these cells change throughout the menstrual cycle and change again during pregnancy ***(Diedrich et al., 2007).***

In the early 1950s, Noyes and co-workers ***(Noyes and Haman, 1953; Noyes et al., 1950)*** examined the histological features of endometrial biopsies taken during 8000 spontaneous cycles in 300 women. By associating histological changes with natural changes in basal body temperature, they were able to link distinct histological patterns to particular time points during the menstrual cycle. The criteria for endometrial dating that resulted from this work have since remained the gold standard approach for evaluating endometrial responsiveness and detecting endometrial abnormalities.

The classical work describing the dating of the endometrium, by ***Noyes et al. (1950)***, dates from more than 50 years ago. Interestingly,this article was the most cited one in infertility literature for a long time ***(Key and Kempers, 1987)***. Several textbook recommendations on the evaluation of the infertile couple include luteal phase assessment of the endometrial histology. However in the recent years, new and updated methods to evaluate the endometrium have been

proposed making the classical criteria of Noyes somewhat outdated ***(Acosta et al., 2000; Lessey et al., 2000).***

In some cases, the menstrual cycle date, which is based by the pathologist on Noyes' criteria, lags behind the actual cycle date. When this lag is of more than 2 days, the endometrium is considered to be 'out of phase'. Patients diagnosed with an 'out of phase' endometrium were counselled to treat this condition by hormonal means. The original Noyes' criteria compared endometrial dating with the estimated day of ovulation based on an increase in basal body temperature. This estimate was later shown to be accurate only in 77% of patients. In comparison, a better accuracy can be obtained by LH surge detection or by ultrasound demonstration of ovulation 85 and 96%, respectively ***(Shoupe et al., 1989).***

More recently, it was shown that the prevalence of an 'out of phase' endometrium in the fertile population is extremely high (49%). In fact, these investigators found that fertile women were more likely to have an 'out of phase' endometrium than infertile women ***(Coutifaris et al., 2004).*** Moreover, the Noyes' criteria, even when examined in normal fertile women, lack the precision to be used to accurately date the endometrium ***(Murray et al., 2004).*** It can thus be concluded that histological evaluation adds little significant information pertaining to the

treatment of the infertile couple. More significant markers will surely replace histological criteria in the near future.

2-Pinopods:

Pinopods are bleb-like protrusions found on the apical surface of the endometrial epithelium ***(Usadi et al., 2003).*** These structures are several micrometers wide and project into the uterine lumen above the microvilli level. They were first described in mice ***(Nilsson, 1958)*** and later in human endometrium ***(Achache and Revel, 2006; Murphy et al., 1987).***

The term 'pinopod', from the Greek 'drinking foot', signifies their pinocytotic function in the mouse ***(Enders and Nelson, 1973).*** Nevertheless, this pinocytosis capacity was not detected in human ***(Adams et al., 2002).*** Electron microscopy is the major tool used to observe these structures ***(Martel et al., 1987&Johannisson and Nilsson, 1972)***. However, use of light microscopy has been proposed so as to facilitate their detection ***(Develioglu et al., 2000)*** **(Figure 1).**

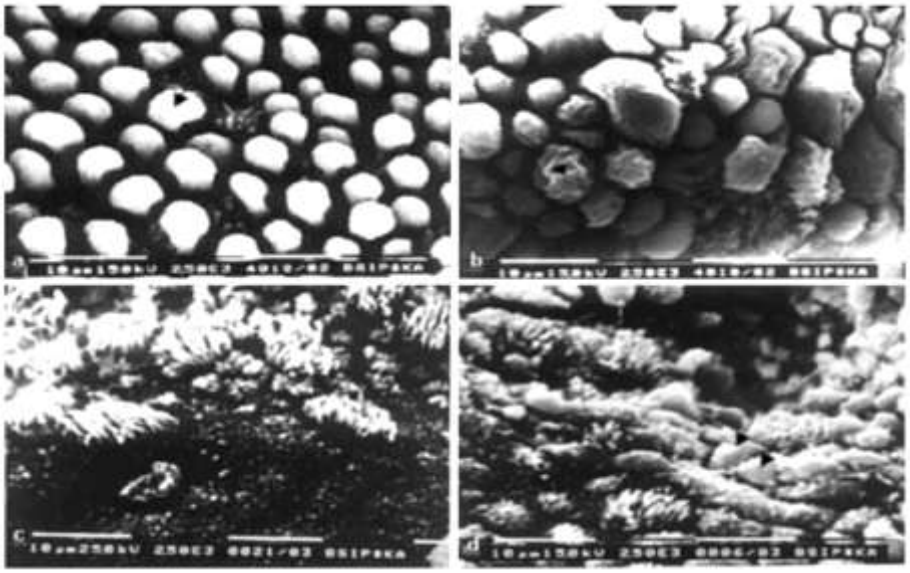

Fig.(1): Scanning electronmicroscopy pictures of apical surface of the uterine luminal epitheliumin endometrial samples during the window of implantation from: (a) a fertile woman with fully developed pinopods (arrow) covering almost the entire endometrial surface, (b) a fertile woman showing regressing pinopodes (arrow head) and few fully developed pinopods covering almost the entire endometrial surface, (c) an infertile woman showing complete absence of pinopods.Many ciliated andmicrovillous cells are seen, (d) an infertile woman showing fewisolatedpinopods (arrows) with smooth surface.Ciliated andmicrovillous cells are clearly visible ***(Makker and Sing, 2006).***

Pinopod expression is limited to a brief period of maximum 2 days in the menstrual cycle corresponding to the putative window of implantation ***(Aghajanova et al., 2003).*** Others have detected that pinopods are present throughout the mid to late-secretory phase, however, displaying cycle-

dependent morphological changes. This suggests that morphology, rather than pinopod presence or absence, is of great significance ***(Usadi et al., 2003).***

The pinopod-regulated expression pattern throughout the menstrual cycle advocates their use as markers of implantation. Pinopods appear to be progesterone dependant. Association between mid-luteal increase of progesterone level and the first appearance of pinopods throughout the menstrual cycle was noted ***(Usadi et al., 2003; Stavreus- Evers et al., 2001).***

Moreover, *HOXA-10*, a homeobox gene whose expression is necessary for endometrial receptivity to blastocyst implantation, has an essential role in pinopod development. Indeed, blocking *HOXA-10* expression dramatically decreases the number of pinopods. *HOXA-10* illustrates a dual role in the endometrium by regulating both endometrial stromal cell (ESC) proliferation and epithelial cell morphogenesis ***(Bagot et al., 2001).***

Although the role of pinopods remains unknown, it seems that they are the preferred sites of embryo–endometrial interactions. Blastocyst attachment was shown to occur onto the top of endometrial pinopods ***(Bentin-Ley et al.,1999).*** Hypothetically, the receptors required for blastocyst adhesion are located on the pinopod surface. Endometrial pinopods

development is associated with the mid-luteal phase increased expression of leukaemia inhibitory factor (LIF) and its receptor ***(Aghajanova et al., 2003),*** progesterone ***(Stavreus-Evers et al., 2001)*** and integrin αVβ3 ***(Lessey et al.,2000).***

The detection of pinopods during the mid-secretory phase may be extremely useful for the assessment of endometrial receptivity to optimize implantation rates ***(Achache and Revel, 2006).***

II) Process of implantation:

1-Decidualization:

In order for implantation to occur, endometrium has to be changed into decidua. This process consists in modifying endometrial stromal cells, uterine glands and vessels, as well as the population of uterine immune cells ***(Salamonsen et al ., 2003).***

In humans, unlike other species decidualization is independentof the blastocyst's presence in the uterine cavity and begins in the late secretory phase of the menstrual cycle. It is evoked by progesterone, as well as by regulatory agents able to enhance cyclic AMP (cAMP) levels ***(Brosens et al., 2002).***

Decidualization continues in pregnancy, and it is thought to regulate subsequent trophoblast invasion and placenta formation by altering the expression of regulatory factors such