INTRODUCTION

Bacterial vaginosis (BV) is a vaginal flora disorder in up to 30% of women of childbearing age ***(Livengood et al., 2009)***.

Bacterial vaginosis appears when the normal vaginal flora, mainly composed of lactobacillus spp, decreases to be replaced by other micro-organisms, such as Atopobium vaginae, Gardenella vaginalis and Mycoplasma hominis as well as other anaerobes ***(Leitich et al., 2003).***

Bacterial vaginosis can be found in 6-63.7% of pregnant women ***(Guaschino et al., 2003)*** and would be responsible for two fold increase in obstetrical morbidity risks of early miscarriage ***(Ralphet al., 1999).***

There was evidence that the pathogenic effects of BV may not confined to complications during pregnancy, but may also interfere with embryo implantation. BV had been reported to be associated with a significant increased risk of miscarriage in the first trimester in women undergoing IVF, independent of other risk factors ***(Ralph et al., 1999)***.

The mechanism by which BV may lower IVF success rate is unclear. The term 'bacterial vaginosis' rather than 'vaginitis' is used, because of the absence of associated inflammation.

However, there is evidence for an inflammatory response to BV.

It had been shown that almost half of patients with symptomatic BV showed histopathological evidence of plasma cell endometritis ***(Korn et al., 1995)***.

In addition, a number of studies had shown correlations between the concentrations of cytokines in both vaginal fluid and cervical mucus and the presence of BV ***(Cauci et al., 2003; Hedges et al., 2006; Mattsby-Baltzer et al., 1998; Spandfor et al., 2001; Platz-christensen et al., 1993; Strum- Ramirez et al., 2000)***.

Significantly diminished pregnancy rates after IVF had been reported when endotoxin concentrations >200pg/ml were found in menstrual effluent ***(Kamiyama et al., 2004)***.

Successful implantation appeared to be associated with predominance of anti-inflammatory cytokines ***(Wegmann et al., 1993)***.

In addition, studies had shown that subjects who tested negative for bacterial contamination had approximately 50%higher pregnancy rates than subjects who tested positive for bacterial contamination, resulting from the embryo transfer catheter tip. An increased risk of pregnancy loss prior to the sixth week of gestation was also reported in women undergoing

in vitro fertilization treatment while having bacterial vaginosis (BV) ***(Liversedge et al., 1999)***.

It had been reported that metronidazole given at oocyte recovery in IVF- embryo transfer cycles may reduce bacteria on the transfer catheter and therefore improve the pregnancy rate ***(Egbase et al., 1999)***.

AIM OF THE WORK

Research hypothesis:

Bacterial vaginosis infection decreases pregnancy rate after IVF/ICSI cycles.

Research question:

Does bacterial vaginosis infection decrease pregnancy rate after IVF/ICSI cycles?

Primary outcome:

Biochemical pregnancy rate.

Secondary outcome:

Clinical pregnancy rate.

Chapter 1

IVF/ ICSI

Introduction:

Edwards and Steptoefirst described the technique for in-vitro fertilization (IVF) and embryo transfer (ET) in 1976 and the subsequent births of two normal babies in 1978 ***(Steptoe and Edwards, 1978)***. Since then, the success rate of the system has been improved (to 30%) by the use of fertility drugs to provide more oocytes and prematuration to mature the oocytes before fertilization ***(Trounson et al., 1981).*** The techniques are now used in 53 countries throughout the world. In 1993, the results of 492 units from all over the world were collected from national surveys and registers. Since 1985, more than 53 635 women had been treated and 34 316 babies had been born from 224473 treatment cycles, following more than 160518 transfer cycles. Only about 65-75% of all resulting pregnancies attained live births. The remainder ended with spontaneous abortions (26%), or ectopic pregnancies (5.54%). The multiple pregnancy rate (22%) was higher than the normal population and contributed to higher rates of preterm deliveries and perinatal mortality. No increased incidence of chromosomal alterations and malformations were noted during the years (2.25%) ***(Gabort Kovacs, 1997)***.

Since the birth of the first IVF baby, tremendous developments have occurred regarding the indications for

assisted reproductive technology (ART). For example, the dramatic development concerning male infertility which initially was considered to involve a small fraction of patients benefiting from IVF, now with the development of intracytoplasmic sperm injection (ICSI), involves up to 35% of started cycles ***(Gabort Kovacs, 1997).***

However, a substantial group of patients still remain unhelped. There is a reduction in success rates in women between the ages of 35 and 39 years, and a further reduction in women older than 40 years of age. There is also doubt about the cost-benefit of ART versus conventional therapy for subfertility. In women with unexplained infertility, hMG super ovulation therapy for subfertility. In women with unexplained infertility, hMG super ovulation treatment is as successful, less expensive, and carries a smaller risk than the surgical approach of ART. However, IVF is more expensive than three cycles of hMG and intrauterine insemination ***(Gabort kovacs, 1997)***.

The indications for treatment were broadened over the years and the procedure became a final step for diagnosis and treatment of unexplained infertility.

Use of gonadotrophin-releasing hormone agonists (GnRHA) has improved pregnancy rates, reduced blood sampling and prevented natural ovulation. The disadvantages of stimulated cycles include higher risks of multiple pregnancy, hyperstimulation and side effects of the drugs ***(Gabort Kovacs, 1997)***.

IVF/ET has become a relatively safe procedure but should be used only when indicated. To obtain the best results, quality control should be regulated by professional and/or public associations and further research is necessary in order to improve success rates.

Natural cycles or immature egg collection (IOC) may become alternatives to the use of the stimulated cycle. Multiple pregnancies may be reduced, by reducing the number of eggs or embryos transferred. Embryo freezing has made an important contribution to overall pregnancy rates by enabling patient to use excess eggs and embryos. The social and legal concerns resulting from the use of frozen embryos requires new ethical and legal consideration ***(Gabort Kovacs, 1997)***.

Donor eggs have made a contribution to achieving pregnancy in women with absent or repeated poor-quality eggs and have increased the chance of conception in women over the age of 40. Micromanipulation of sperm and eggs has enabled fertilization and conception when sperm are defective in quantity and quality ***(Gabort Kovacs, 1997)***.

In summary, ART has developed over a decade to become useful for couples with infertility which cannot be cured by simple treatments. The birth rates are comparable to natural conception and the incidence of congenital malformations is not increased. The costs and complexities of treatment have been reduced, which in turn has reduced the

stress and social inconvenience of therapy. Problems related to the birth risk of multiple pregnancy and the use of the stimulated cycle are being reduced as new techniques for severe male infertility and the detection of genetic abnormalities in the embryo have been introduced.

Indications:

Tubal Infertility:

Tubal infertility is defined as persistent bilateral tubal obstruction, absence of tubes or tubal damage which has resulted in a period of infertility of more than 12 months' duration. Possible therapy of tubal infertility includes hysterosalpingogram, chromatography at the time of laparoscopy, selective transcervical tubal cannulation and falloposcopy. Each of their effects is restricted to the mechanical defect that may involve the tubal pathology ***(Gabort Kovacs, 1997)***.

Tubal surgery, when indicated, allows for natural conception and more than one pregnancy can be achieved as a result ***(Smalldrudge and Tait, 1930)***. It should be considered the first-line treatment for proximal tubal disease, reversal of sterilization and fimbrial adhesions. In vitro fertilization should be offered to those with severe distal tubal disease and severe endometriosis. It may also be indicated in patients who have not conceived within one year following microsurgery. In patients with patent tubes following microsurgery, gamete

intrafallopian transfer may be performed. More clinical experience can solve the dilemma of whether it is beneficial to perform GIFT or to offer IVF primarily to the patient ***(Gabort Kovacs, 1997)***.

Between January 1987 and December 1990 at the Jones Institute for Reproductive Medicine, the most common indications for IVF/ET were tubal factors (57%) ***(Seard and Jones, 1992)***.

Much of the discussion about the management of tubal disease has centered on the cost of ART versus tubal surgery. Most authors agree that the cost of IVF is less than, or the same as, that of tubal surgery. ART is the preferred method for women suffering from multiple tubal obstruction, after bilateral salpingectomy, or with extensive and dense pelvic adhesions ***(Gabort Kovacs, 1997)***.

Check et al. (1994) compared the cumulative probability of pregnancy after multiple IVF cycles by age and case of infertility. The three-month cumulative probabilities of pregnancy based on life-table analysis were 33% in women with tubal factor who were younger than 35 years of age, 25% in women with tubal factor who were older than 35 years of age, 30% for women with multiple factors who were younger than 35 years of age, and 14% for women with multiple factors who were older than 35 years of age. This study demonstrates

the significance of the effect of age and infertility factors on pregnancy and delivery rates.

Male Infertility:

Male infertility has become as common as tubal factors as an indication for IVF/ET. Initially, male subfertility was treated using GIFT when couples had not responded to any other method of treatment, including intrauterine insemination, and patent tubes were present ***(Wiedermann and Hepp, 1989)***. However, the results show that the pregnancy outcome after GIFT in couples with severe male infertility is significantly lower than that following GIFT with normal sperm.

With the establishment of ICSI for couples with male infertility, most causes of male infertility can now be treated. The majority of infertile males have oligospermia and/or low sperm motility and are subfertile rather than sterile. In men with azoospermia, sperm cells obtained from the epididymis or by testicular biopsy may prove satisfactory for the ICSI technique ***(Gabort Kovacs, 1997)***.

The exceptional results of subzontal injection of the oocyte (SUZI), and more particularly ICSI, now raise the prospect of pregnancy being possible even when there are low numbers of sperm in the ejaculate, and bring to treatment focus some azoospermic men and men without antegrade ejaculate. The advance in clinical results from SUZI and ICSI over earlier zona

drilling and zona binding techniques appears to be giving better implantation potential to the embryos ***(Gabort Kovacs, 1997)***.

Technical factors critical for achieving high rates of fertilization and pregnancy include the use of standardized ICSI pipettes, the immobilization of sperm before injection, and the aspiration of a minimal amount of ooplasm before reinjection with the sperm. Intracytoplasmic sperm injection is superior to other micromanipulation methods for alleviating male infertility. Recently, it has been shown to be superior in terms of fertilization rates and pregnancy rates when compared with SUZI. However, there has been some concern regarding the abnormal fetal karyotypes. ***Van Steirteghem et al. (1993)*** have reported that a single spermatozoon was injected into the ooplasm of oocytes with a fertilization rate of 64.2%, and this was not influenced by sperm morphology or motility. Total and clinical pregnancy rates of 49.6% and 39.2%, respectively, per ET were reported. This new development arises skepticism regarding the applicability of conventional semen parameters. If normal embryos can be produced by ICSI, doubts regarding such theories as a natural selection process and the correlation of sperm head morphology and quality of the DNA arise.

In conclusion, only ten years after ***Yovich and Stanger*** stated in ***1984*** that only a small fraction of oligospermic men benefited from IVF, the results of this treatment for male infertility had improved dramatically.

Endometriosis:

The aetiology of infertility in endometriosis is unclear, and a multitude of factors are involved. In advanced (Stage III and IV) endometriosis, endometriosis and pelvic adhesions distort pelvic anatomy and mechanically interfere with the reproductive process. In stage I and II disease, the mechanicism of infertility is less clear. These effects are relative and individually variable, and some women with endometriosis have no infertility problems ***(Gabort Kovacs, 1997)***.

A variety of mechanisms through which limited endometriosis may lower fertility have been postulated:

- Ovulatory dysfunction, the prevalence of which would seem to be similar in endometriosis and in the infertile population in general.
- Alterations in gamete/embryo transfer.
- Anti-fertility effects of the peritoneal fluid, which may affect sperm motility/survival, fertilization, gamete interaction and early embryo development.
- Peritoneal macrophages causing increased sperm phagocytosis, decreased motility and impaired ability to penetrate the egg.
- An anti-implantation effect related to evidence for antigen-antibody reaction in the uterine cavity of women with

endometriosis which may be the mechanism of infertility and recurrent miscarriages.

Most of the mechanisms implicated in endometriosis should be corrected by IVF/ET. Aspiration of the eggs with ultrasound-guided needles, in-vitro fertilization, early embryonic development in the laboratory and embryo transfer into the female reproductive system should correct problems caused by ovulatory dysfunction, abnormal fertilization, failure of early embryonic development. One would, therefore, expect IVF/ET pregnancy rates to be comparable in patients with and without endometriosis ***(Gabort Kovacs, 1997)***.

There is no agreement in the literature regarding the benefits of medical pretreatment of endometriosis before the IVF cycles. The most common medical pretreatments were either danazol or GnRHA.

Ovulatory Disorders:

Disorders of ovulation resistant to treatment by ovulation induction with either clomiphene citrate or urinary gonadotrophins may respond to IVF/ET. The most common disorder of ovulation which may prove resistant is PCOS. The presenting symptom of patients with PCOS is often infertility due to chronic oligo-ovulation or anovulation, and the restoration of ovulatory function assumes paramount importance ***(Gabort Kovacs, 1997)***.

Clomiphene citrate is the first line of treatment for chronic anovulation that accompanies PCOS. However, if it fails, then conventional gonadotrophin therapy is indicated. Unfortunately, this therapy is associated with an increased spontaneous abortion rate, multiple gestation rate and OHSS. Although today's readily available monitoring technology (ultrasonography) and rapid serum oestrogen measurements make gonadotrophin therapy safer in terms of multiple gestation and OHSS, they are both time intensive and expensive and these complications lead to a high rate of cycle cancellation ***(Gabort Kovacs, 1997)***.

The use of IVF/ET in patients with PCOS enables:

- Controlled ovarian stimulation in conjunction with GnRHA and down regulation ***(Hombergefai, 1993)***.
- Aspiration of all ovarian follicles present.
- The option of freeze/thawing all resultant embryos and subsequent transfer in a non-stimulation cycle.
- The resultant decrease in the incidence of OHSS.

In summary, IVF/ET is being increasingly used in association with ovulatory disorders and in particular PCOS. However, despite the high pregnancy rate from transfer of embryos, there is a high first trimester abortion rate as well ***(Gabort Kovacs, 1997)***.

Unexplained Infertility:

Unexplained infertility can be defined to include those couples with more than two years of infertility with no abnormalities on repeated investigation of the fallopian tubes, ovulation, luteal phase, cervical mucus, semen, semen-mucus interaction or intercourse. However, it should be noted that there are many couples who have minor abnormalities but no adequate explanation for their inability to conceive. The duration of the infertility and the age of the patient are particularly important ***(Gabort Kovacs, 1997)***.

It should also be noted that a large number of patients enter IVF programmes as "idiopathic infertility" but that during subsequent investigations and treatment, the cause of the infertility may become apparent. Therefore, IVF/ET has resulted in the diagnosis and treatment of idiopathic infertility.

Many empirical modalities have been suggested for the treatment of couples with unexplained infertility. These include IUI, steroid and antibiotic therapy, bromocriptine, ovulation induction, combined treatments and ART ***(Navot et al., 1988)***. The ESHERE muticentre trial was designed to compare the effectiveness of superovulation alone with superovulation with either IUI, GIFT, IVF or intraperitoneal insemination. The pregnancy rates obtained in the trial were in excess of rates reported for untreated couples, and the mean pregnancy rates for the four invasive methods were similar. However, the