Introduction

Infertility is defined as one year of frequent, unprotected intercourse during which pregnancy has not occurred ***(Alaina et al., 2007)***.

Infertility in many couples has multiple etiologies; however, in approximately 15-17% of couples, no reason for infertility is found and the infertility is defined as unexplained ***(Aboulghar et al., 2001)***.

Unexplained infertility refers to a diagnosis in whom standard investigations including semen analysis, tests of ovulation and tubal patency tests are normal ***(Siristatidis and Bhattacharya, 2007)***.

The average incidence of unexplained infertility has been reported to be approximately 15%. The incidence varies from 0% to 37%. This variation may be attributed to selection bias in referral-based infertility practice ***(Balasch, 2000)***.

The utility of anything other than basic tests of semen quality, ovulation and tubal patency in the diagnosis and management of infertility has yet to be proven ***(Steures et al., 2006)***.

Protein C is a 62-kD, vitamin K-dependent glycoprotein synthesized in the liver. It circulates in the blood as an inactive

zymogen at a concentration of 4 μg/mL. Its activation into the serine-protease-like enzyme, activated protein C (APC), is catalyzed by thrombin when it is bound to the endothelial proteoglycan thrombomodulin (TM) ***(Dahlbäck, 2008).*** This reaction is greatly stimulated by the endothelial protein C receptor (EPCR), which binds protein C and brings it in close proximity with the thrombin/TM complex ***(Dahlbäck and Villoutreix, 2005)***.

Normal protein C activity is between 60 to 180% of normal. Deficiency is certain if protein C is repeatedly below 60% ***(Rai and Regan, 2000)***.

Protein C is a major component in anticoagulation in the human body. It acts as a serine protease zymogen: APC proteolyses peptide bonds in activated Factor V and Factor VIII (Factor Va and Factor VIIIa), and one of the amino acids in the bond is serine ***(Mosnier and Griffin, 2006).***

Protein C pathway abnormalities are the most frequent biological risk factors for venous thromboembolism (VTE) and they can be demonstrated in up to 30% of white patients with thrombophilia ***(Seligsohn and Lubetsky, 2001).***

The majority of thrombophilias associated with placental vascular complications during pregnancy have been related to the protein C pathway ***(Brenner and Kupferminc, 2003)***.

Thrombophilia seems to be more frequent than expected among the healthy population, and could impair implantation in some subgroups of infertile women ***(Bellver et al., 2008).***

Thromboembolism is the leading cause of antepartum and postpartum maternal mortality. The hemostatic system appears to play an important role in both the establishment and maintenance of pregnancy. Several authors have found an association between thrombotic predispositions and recurrent pregnancy loss (RPL). The proposed mechanisms for fetal loss include inhibition of the thrombolytic system, placental thrombosis, placental infarction, abnormal prostacyclin metabolism, and direct cytotoxic effects ***(Rai and Regan, 2000).***

Protein C deficiency is now speculated to be associated with both non-recurrent and recurrent first, second and third trimester miscarriages, IUFD, intrauterine growth retardation (IUGR), placental abruption and preeclampsia ***(Sugiura, 2005).*** Activated PC resistance predisposes to early recurrent fetal loss ***(Rey et al., 2003).***

Aim of the Work

This study is designed to find out a possible relationship between unexplained infertility and protein C activity.

Unexplained Infertility

Introduction and Definitions:

Infertility is defined as the inability to conceive after one year of sexual intercourse without using any contraceptive means ***(Speroff et al., 2004).***

About one of seven couples in developed countries and one of ten couples in developing countries experience infertility ***(Duckitt, 2002).***

A couple is diagnosed with unexplained infertility if the results of infertility workup investigations are normal because the diagnosis of unexplained infertility ideally includes only couples with real but subtle defects in reproductive function, many investigators use a longer duration of involuntary infertility such as 2 or 3 years to diagnose unexplained infertility ***(Kim and Hornstein, 1997)***.

Unexplained infertility does not mean there is no physical explanation for the infertility, but that is just, medical tests have not identified any specific problems ***(Randolph, 2000; Cahill and Wardle, 2002)***.

A quarter of infertility range (25%) cannot be explained because of: Current tests are not perfect in finding all problems.

- The problem preventing pregnancy is not covered by the usual range of tests for assessing infertility.
- There are causes which are not yet understood by scientists.
- There are some factors which might have effect, but no reliable supporting tests exist ***(Cahill and Waardle, 2002).***

Although infertility has many known causes, some couples are still classified as having unexplained infertility (UI) because the underlying mechanism(s) is never found. Some of the infertile couples undergo unsuccessful IVF attempts after the replacement of several embryos and are thus defined as having implantation failure (IF). Moreover, they may experience early pregnancy losses in both natural conception and assisted reproduction cycles, which go unnoticed by the woman ***(Simon et al., 1999).***

In addition, there is another subgroup of infertile couples that conceive but lose the pregnancy at some point, and these are classified as having recurrent spontaneous abortion (RSA). These three conditions are frustrating, and may have common mechanisms by which the embryo does not implant in the uterus, or fails to continue normal development to term. Some of these common mechanisms have been clearly identified, whereas others remain mere speculation ***(Christiansen, 2006)***.

Incidence:

About one of seven couples in developed countries and one of ten couples in developing countries experience infertility ***(Duckitt, 2002).***

The incidence of unexplained infertility in the 1950s was greater than 20% ***(Southam, 1960)***.

In Britain, male factor infertility accounts for 25% of infertile couples, while 25% remain unexplained. 50% are female causes with 25% being due to anovulation and 25% tubal problems ***(Khan et al., 2005).***

Although the prevalence has been stable during the past 50 years, there is a shift in etiology and in patient population age range ***(Speroff et al., 2004)***.

Table (1): Incidence of most frequent causes of infertility

Cause of infertility	%
Ovulatory disorder or anovulation	27-30
Tubal factor	20-22
Abnormal semen parameter	24-40
Unexplained	15
Endometriosis	5
Uterine or cervical factor	4-5

(Collins et al., 1995; Kelly et al., 2001)

Possible Etiologies

l- Hormonal disorder:

Subtle ovulatory dysfunction:

Ovulation is of fundamental importance to survival of the species. Extrusion of an oocyte containing the maternal genetic material from the ovary starts the process which results eventually in a new individual. It is essential therefore that the appropriate numbers of oocytes are ovulated and that these oocytes should be capable of being fertilized and forming a normal embryo ***(Baird and Mitchell, 2002).***

Subtle ovulatory dysfunction has also been postulated as an etiology of unexplained infertility. A history of regular menstrual cycles provides an indication that ovulation is most likely taking place. Nevertheless, current diagnostic tests for ovulation (for example, basal body temperature shift, day 21 serum progesterone, and endometrial biopsy in the late luteal phase) provide only indirect evidence of ovulation and cannot confirm the actual release of the oocyte ***(Paulson, 1997).***

There is now substantive evidence that a high proportion of the oocytes ovulated are developmentally incompetent, even when folliculogenesis appears to proceed normally. The percentage of defective oocytes increases strikingly with increasing age and is the main reason for the decline in fecundity in older women. Although there is extensive interaction between the oocyte and the somatic cells during

folliculogenesis, there is little evidence that the occurrence of ovulation depends on the developmental competence of the oocyte. Therefore, reproductive quality control is exerted at fertilization, implantation and fetal development rather than at ovulation. Unfortunately, while the number of oocytes developing in IVF cycles may reflect oocyte quality, direct diagnostic tests do not exist that can allow an assessment of oocyte quality ***(ESHRE, 2004).***

Luteal-Phase Defect:

LPD is said to exist if discrepancy of > 2 days is noted. This LPD leads to diminished endometrial receptivity and consequent lack of embryo implantation ***(Speroff et al., 2004).***

Because an embryo is less likely to become implanted on an endometrium that is thought to be less receptive, LPD has been postulated as a cause of unexplained infertility ***(Speroff et al., 2004).***

The diagnosis of LPD should be considered in women with normal cycles and unexplained infertility, finding of an out of phase biopsy in approximately 20% to 30% of normal cycles and repetitive lags in more than one normal cycle of approximately 5% ***(Batista et al., 1993).***

Extra ovarian influence on follicular function:

When ovulation appears normal, there seems to be no reason to attribute infertility to endocrine disorders. There is evidence of impaired fertilizing ability of oocytes in women

undergoing IVF treatment who have raised LH levels that could be attributed to as a cause of unexplained infertility**.** Hyper-prolactinemia has been associated with abnormal luteal function and also with unexplained infertility in women with apparently normal luteal phase ***(Balasch et al., 2000).***

II-Pelvic Abnormalities:

Prospective studies appear to have clearly demonstrated the substantial importance of even minimal endometriosis which has been shown to be associated with impaired fertilization ability of oocyte and presumably impaired follicular function. Changes in the intraperitoneal environment leading to an inflammatory process in the absence of visible abnormalities have been suggested as being causal in some cases of unexplained infertility ***(Cahill and Wardle, 2002).***

Many investigators have pointed towards similar patient profiles in women with mild cases of endometriosis and UI. Suggestions have, therefore, been made that endometriosis may be under diagnosed and that UI may in many cases, represent a non-visible and/or only microscopic precursor stage of endometriosis ***(Nezhat et al., 2005).***

III-Sperm Disfunction and Antisperm Antibodies:

It is now recognized that not all sperm antibodies impair fertilization. In addition, low levels of sperm antibodies have been shown to have poor prognostic value in predicting fertility. Therefore, diagnosing someone as having antisperm

antibodies in general does not have clear prognostics significance ***(Szczepanska et al., 2001).***

One defined cause of defective sperm function is oxidative stress created by excessive generation of reactive oxygen species (ROS) by the sperm and/or the disruption of antioxidant defence systems in the male reproductive tract. Excess free radical generation may involve defective spermiogenesis with high levels of cytoplasmic retention and consequent ROS generation. The consequences of such oxidative stress include a loss of motility and fertilizing potential and the induction of DNA damage in the sperm nucleus ***(Aitken and Krausz, 2001)***. The causes and consequences of oxidative damage to the DNA in the sperm nucleus are still not known with certainty. The available evidence suggests that, in addition to a reduced chance of spontaneous pregnancy ***(Loft et al., 2003)***, there will be a reduced chance of a live birth following IVF/ICSI ***(Evenson and Jost, 2000; Larson et al., 2000)***

The analysis of data collected by the spermiograms showed that semen quality could benefit from lifestyle changing. Smokers and drinkers (for both coffee and alcohol) had lower sperm volume, lower sperm motility and vitality when compared to the others. This finding is important for the management of patients with varicocele, suggesting that lifestyle changing could avoid surgery ***(Zampieri et al., 2008).***

IV- Uterine receptivity:

The implantation process, currently thought to be the most critical step in achieving a successful early pregnancy, remains one of the most important unsolved processes in reproductive medicine. It depends on uterine-dependent and embryo-specific events, which need to be critically coordinated. Early embryo signaling following a maternal hormonal or cytokine-mediated preparation phase seems to be involved in stages immediately before, during and just after the opposition step to permit adequate proliferation of the Strom studies on the uterine luminal environment at the time of oocyte retrieval and on monitoring the endometrium using three-dimensional ultrasound associated with digital technology and cytokine quantification by real-time PCR during the implantation window in an IVF/ICSI population. There is an accumulating body of data which strongly suggests that both implantation and uterine receptivity are controlled, primarily, though not exclusively, by locally acting growth factors and cytokines, some under steroid control. Some specific cytokines (II -12, IL-15 and IL-18) in the luminal environment and in the endometrium allow a distinct pattern of abnormal uterine receptivity. The identification of these distinct pattern of abnormal uterine receptivity and of the mechanisms leading to the abnormal angiogenesis before implantation strongly suggest that no single therapeutic scheme can correct all cases of implantation failure and should be adapted for each patient especially in the case of unexplained infertility ***(Lédée et al., 2007).***

V-Genetics Causes:

Li and his colleagues examined the endometrial expression of Homeobox A10 (HOXA-10) gene in normal female women and in women with unexplained infertility. These authors found a higher incidence of aberrant HOXA-10 expression in patients with unexplained infertility. The study suggested that altered development of the endometrium at a molecular level may contribute to the etiology of infertility ***(Li et al., 2002).***

VI-Immunological Causes:

Many investigators have reported a clustering of sub-clinical autoimmune abnormalities in infertile populations. The most convincing evidence comes, however, from the widely reported observation that women with classical autoimmune diseases, even before they reach diagnosis (i.e. become clinically symptomatic) in other words, at pre-clinical stages of their impending autoimmune diseases already demonstrate decreased fecundity. Unexplained infertility may be a reflection of decreased fecundity because of abnormal immune function ***(Siristatidis and Bhattacharya, 2007).***

VII- Cervical Mucus:

The nulliparous cervix is approximately 2 cm in length. Its columnar epithelium is pierced by the ducts of mucous secreting glands which spew forth their contents as a protective barrier preventing bacteria from entering the upper reproductive tract and as a welcome channel to lead sperm into the upper tract. Cervical

mucus is comprised of hyaluronic acid. Micelles which are affected by the hormonal milieu exposed to the glands. Late in the follicular phase, as estrogen rises, the micelles align in a parallel arrangement forming channels to guide the sperm. Under the microscope this can be seen as the classic "fern" pattern of dried cervical mucus. The pH is alkaline and nourishing to the sperm. Indeed, sperm can live in normal cervical mucus for as long as 4 days. The abundant mucus frequent oozes from the cervical os into the vagina to lure the sperm from the ejaculate, while protecting them from the acidity of the vagina. At mid cycle, just prior to and after ovulation the rising progesterone increase the salt content of the mucus, breaking the micelle channels and thickening the consistency of the mucus. The "fern" pattern is no longer seen; the mucus thickens and becomes hostile to sperm and bacteria alike ***(Querleu, 1991).***

Nonetheless, the classic diagnosis of cervical factor of infertility is made by the post coital test. However, the evidence of a poor post-coital associated with infertility is quite poor due to the wide variation in techniques and results. So, thin or absent mucus with any moving sperm on the day of urinary LH surge is strongly suggestive of cervical factor of infertility ***(Oei et al., 1995).***

VIII-Occult infection:

Patients with tubal factor of infertility often have a history of a pelvic infection, endometriosis, or previous abdominal or pelvic surgery. However, patients are unable to

clearly identify a source for their tubal adhesions. For example, a patient who experienced a chlamydial infection may have attributed the lower abdominal pain, fever and cramping to a gastrointestinal viral infection and often cannot recollect the time of infection ***(Oei et al., 1995).***

IX-Psychological Causes:

Collins and his colleagues using psychological and biochemical (prolactin) assessments of stress, reported the identification of a subgroup of unexplained infertility patients who were unusually stressed, and attempted to reduce stress levels within this subgroup by relaxation therapy and by inhibiting prolactin rise with bromocriptine ***(Collins et al., 1995).***

Thrombophilia and hyperhomocysteinemia:

Di Micco and his colleagues suggested thrombophilia as a cause of implantation failure in cases with unexplained infertility particularly secondary infertility due to recurrent pregnancy loss, and for the first time to detected thrombophilia in primary unexplained infertility ***(Di Micco et al., 2004)***

Thrombophilia seems to be more frequent than expected among the healthy population, and could impair implantation in some subgroups of infertile women ***(Bellver et al., 2008).***