INTRODUCTION

The endometrium is a dynamic tissue that responds to changing hormonal signals throughout the cycle. The changes in the endometrial composition are expressed in alteration in gene expression, micro architectural morphological changes as well as in protein and hormone secretion. These factors combine together to construct the "window of implantation" a short period of time during the luteal phase in which the endometrium is receptive ***(Casper and Yanushpolsky, 2016).***

Embryo implantation is a very delicate and well-orchestrated process that is governed by the interaction between several maternal and embryonic factors, ultimately resulting in adherence of the blastocyst to the endometrium ***(Paiva et al., 2011).***

Endometrial thickness is defined as the minimal distance between the echogenic interfaces of myometrium and endometrium, measured in the plane through the central longitudinal axis of the uterine body. Ultrasonographic endometrial thickness ranges from 1 to 4mm in the menstrual phase, 4 to 8mm in the mid-proliferative phase, 8 to 14 mm in the late follicular phase, and 7 to 14mm in the secretory phase ***(Grow and Iromloo, 2006).***

Thin endometrium is associated with a low pregnancy rate. Endometrial thickness 7 mm in the pre-ovulatory phase is widely accepted to be cut-off of thin endometrium ***(Wu et al., 2014).*** Other cut-offs reported in published studies usually range between 5-8 mm ***(Fang et al., 2016).*** Several studies reported on a higher pregnancy rate achieved with a blastocyst stage embryo transfer when endometrial thickness was more than 9 mm ***(Richter et al., 2007).*** Similar endometrial thickness was found to be the cut-off to predict pregnancy following analysis of 1933 IVF cycles ***(Zhao et al., 2012).*** A higher live birth rate with endometrium 9.1-10 mm in donor egg recipients have been reported ***(Dain et al., 2013).***

The incidence of thin endometrium varies according to the chosen cut-off, it was found that the incidence of thin endometrium less than 7 to be 1.49 &2.5% in high responders and poor responders respectively. An association was found between endometrial thickness and age. Thin endometrium was more prevalent among older women; it has been reported in 5 % of women less than 40 years of age and in 25% of 41-45 years old women ***(Bu and Sun, 2015).***

The thickness of the endometrium is dependent on several influences including reproductive age, phase of menstrual cycle, ovarian hormone (estrogen and progesterone) concentration, and endometrial hormone receptor density ***(Paulson, 2011).*** Several mechanisms have been proposed to

explain the underlying pathophysiology of thin endometrium. Intrauterine adhesions, ovarian stimulation with clomiphene citrate (CC), as well as combined oral contraceptive pills, or prolonged use of progesterone have been associated with thin endometrium ***(Casper, 2011).*** One of the most important factors in the pathophysiology of thin endometrium is vascular endothelial growth factor (VEGF), which plays a critical role in angiogenesis. Adequate endometrial development, which is hormonally mediated, is highly dependent on adequate blood supply. It was described that with increased impedance across the radial uterine arteries, there is resultant decrease in VEGF expression and subsequent poor vascular development, resulting in thin endometrium ***(Miwa et al., 2009).***

Description of 'thin endometrium' is still lacking a consensus. Oral contraceptives maintain a very thin, flat endometrium, with a thickness of 1-3mm, even in the late follicular endometrium ***(Grow and Iromloo, 2006).***

Being a hormone dependent tissue, the endometrium proliferates in response to estrogen, which further induces the production of progesterone receptors. As a result, infertile patients who demonstrated thin endometrium, were offered estradiol (E2) remedies, in an attempt to improve endometrial proliferation. Most of the studies regarding E2 treatment in patients with thin endometrium dealt with frozen-thawed

embryo transfer cycles. Moreover, while there are several routes and durations of administration of E2, including per Os, transdermal, intramuscular and vaginal, no compelling advantage for one protocol for endometrial preparation over another, with regard to pregnancy rates, has been established ***(Groenewoud et al., 2013).***

Sildenafil acts as a type 5-specific phosphodiesterase inhibitor hence augments the vasodilatory effects of nitric oxide by preventing the degradation of cGMP. Nitric oxide (NO) relaxes vascular smooth muscle through a cGMP-mediated pathway and NO synthase isoforms have been identified in the uterus. A decade ago, ***Fisch and Sher*** studied the effect of vaginal sildenafil on the endometrial thickness of infertile patients with poor endometrial development, who underwent IVF treatment. They have demonstrated improved uterine artery blood flow and endometrial growth, with higher implantation and ongoing pregnancy rates following vaginal sildenafil administration ***(Fisch and Sher, 2000, 2002).***

AIM OF THE WORK

Compare the effect of sildenafil-estrogen combination to estrogen alone on endometrium thickness in infertile women.

Research question:

In infertile women, is sildenafil/estrogen combination as effective as using estrogen alone in improving endometrial thickness? And outcome of pregnancy?

Research Hypothesis:

In infertile women, Sildenafil and estrogen therapy may improve endometrial thickness like estrogen treatment alone.

Chapter 1

INFERTILITY

Infertility is defined as the inability to conceive after one year of regular unprotected intercourse. The infertility evaluation is typically initiated after one year of trying to conceive, but in couples with advanced female age (> 35 years), most practitioners initiate diagnostic evaluation after an inability to conceive for 6 months ***(Al-Assadi et al., 2012).***

Infertility is a worldwide health problem with one in six couples suffering from this condition and with a major economic burden on the global healthcare industry. Today, infertility is no longer recognized as only a female problem. In fact, the term infertility is a broad term, often loosely used. It actually refers to a range of disorders some of which affect the male, and some the female, and contribute to childlessness in a couple ***(Sharath et al., 2013).***

Infertility is said to be unexplained infertility when a couple does not conceive and no definite cause can be diagnosed after a complete standard evaluation ***(Aboulghar et al., 2002).***

The incidence of unexplained infertility in infertile population ranges from 10–15% and using more invasive diagnostic laparoscopy as criterion and using normal findings, the prevalence may be less than 10% ***(Speroff, 2005).***

Unexplained infertility refers to a diagnosis in couples in whom standard investigation including semen analysis, tubal patency and test of ovulation are normal ***(Siristatidis and Bhattacharya, 2008).***

In female, polycystic ovary disease (PCOD), genital tuberculosis, blockage of tube, fallopian tube defects, endometriosis, obesity, use of certain medication, alcohol consumption and smoking may contribute to the conception problems. Globally, every year 60–80 million couples suffer from infertility while India alone is probably between 15–20 million (25%) ***(Sharath et al., 2013).***

The incidence of infertility is associated with geographic differences. For example, in some western european countries is 12%, while in some west-african communities, infertility rate is around 50% likewise differences are observed both in developed countries, where rates range from 3.5% to 16.7%, as well as in less developed countries, where rates of infertility range from 6.9% to 9.3%. It has also been observed that the causes are related to geographical differences. Especially in western countries, the most common risk factor of infertility is age, while in africa is sexually transmitted diseases ***(Boivin et al., 2007).***

A more recent study puts the prevalence among couples diagnosed as unexplained infertility attending a fertility clinic

to be 21% in women aged under 35 years, and 26% in women over 35 years ***(Maheshwari et al., 2008).***

Causes of Female Infertility

Female infertility may occur when (Figure 1) (Bulun, 2011):

- A fertilized ovum or embryo does not survive once it sticks to the lining of the uterus.
- The fertilized ovum does not attach to the lining of the uterus.
- The ova cannot move from the ovaries to the uterus.
- The ovaries have problems producing ova.

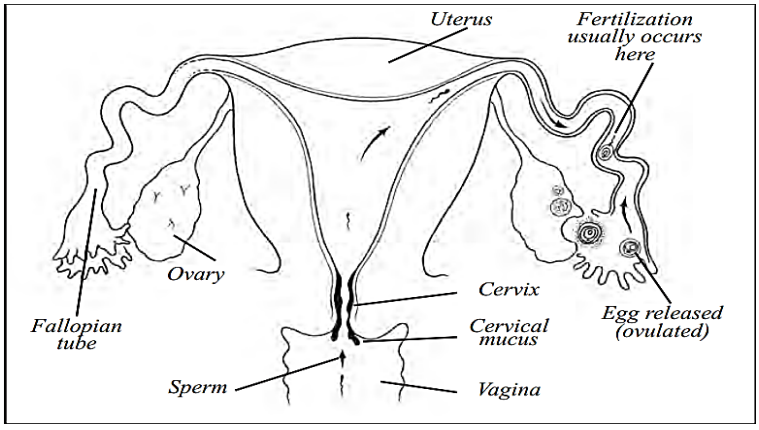

Figure (1): Diagram showing ovulation; fertilization usually occurs in the fallopian tube.

Female infertility may be caused by (Bulun, 2011; Lobo, 2012):

- Autoimmune disorders, such as antiphospholipid syndrome (APS)
- Clotting disorders.
- Diabetes.
- Neoplasia (such as fibroids or polyps) in the uterus and cervix.
- Congenital anomalies of the reproductive tract.
- Excessive exercising.
- Eating disorders or poor nutrition.
- Use of certain medications, including chemotherapy drugs.
- Alcohol intake.
- Obesity.
- Ovarian cysts and polycystic ovary syndrome (PCOS).
- Pelvic infection or pelvic inflammatory disease (PID).
- Scarring from sexually transmitted infection, previous abdominal surgery, or endometriosis (Figure 2).
- Smoking.
- Surgery to prevent pregnancy (tubal ligation) or failure of tubal ligation reversal (reanastmosis).
- Thyroid disease.
- Too little or too much of certain hormones.

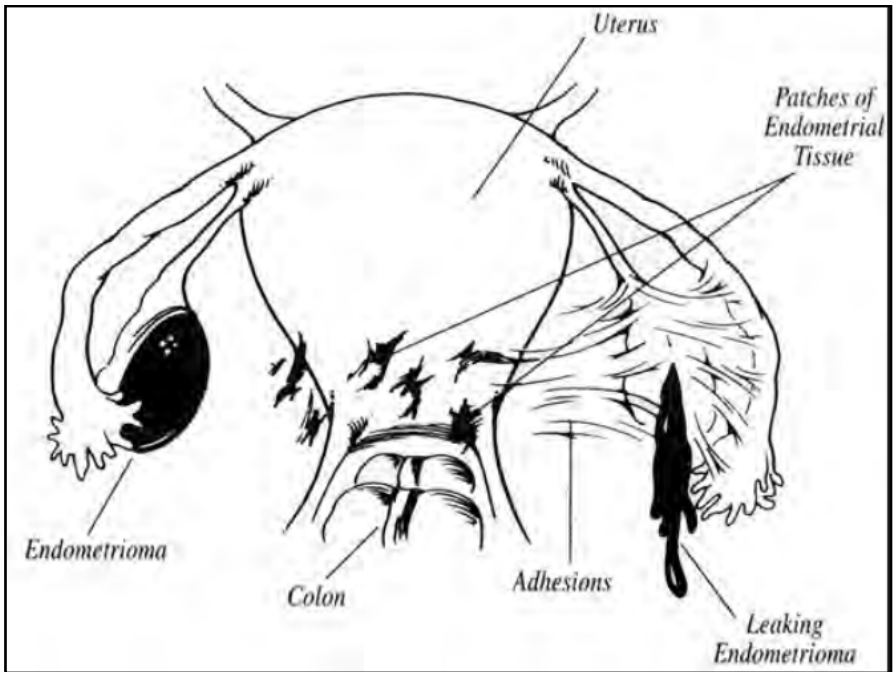

Figure (2): Endometriosis and adhesions.

The National Institute for Health and Clinical Excellence in the UK and the American Society of Reproductive Medicine in the USA have recommended the following essential tests; semen analysis, assessment of ovulation, and evaluation of tubal patency by hystero-salpingogram or laparoscopy (Figure 3 and 4). The place of laparoscopy versus hysterosalpingogram continues to be debated, but it is felt that laparoscopy should be considered when severe endometriosis, pelvic adhesions or tubal disease is suspected ***(Practice Committee of the American Society for Reproductive Medicine, 2012).***

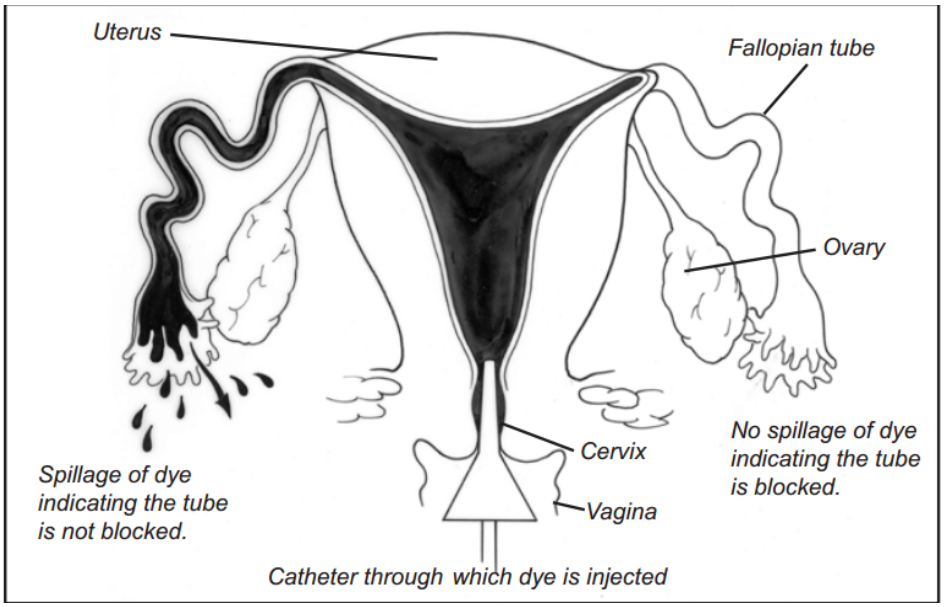

Figure (3): Hysterosalpingogram, a procedure to determine if the fallopian tubes are open or blocked.

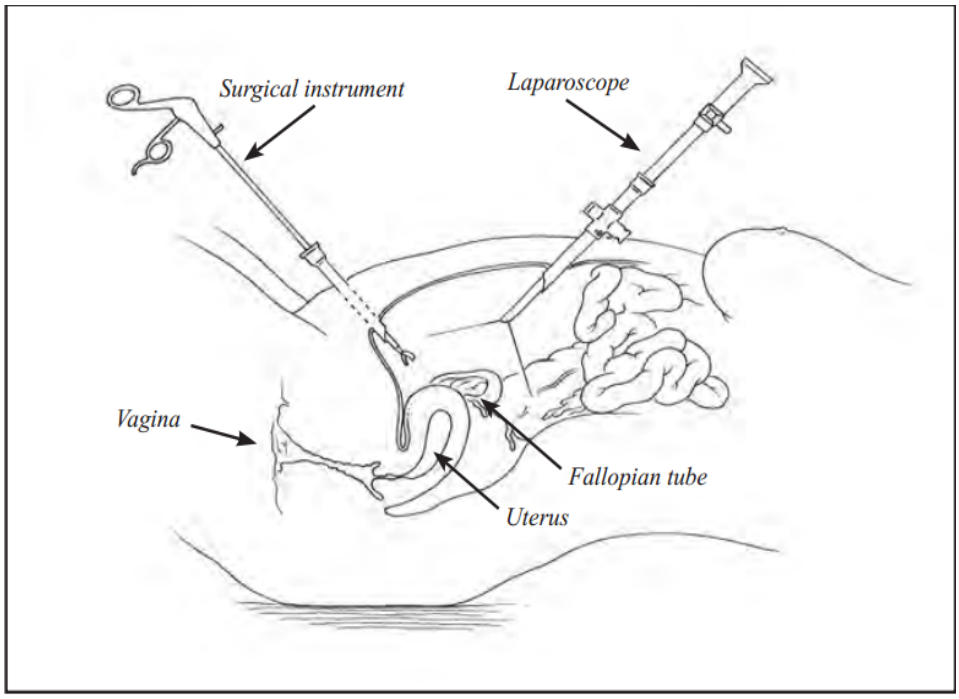

Figure (4): Laparoscopy.

Chapter 2

INDUCTION OF OVULATION

Ovarian stimulation is a way of treatment that might be used as a first line of treatment in young woman with unexplained infertility ***(Peeraer et al., 2018).*** Clomiphene appear to be superior to no treatment or placebo ***(Wang et al., 2000).***

A Cochrane systematic review and meta-analysis of three RCTs (133 randomized women with WHO group 2 anovulation) comparing clomiphene citrate with placebo demonstrated that clomiphene citrate improves ovulation rate per patient (OR = 7.5; 95% CI, 3.24–17.23; P < 0.00001) and pregnancy rate per patient (OR = 5.8; 95% CI, 1.55–21.48; P < 0.009) ***(Brown et al., 2009).***

Clomiphene, a selective estrogen receptor modulator (SERM), having both estrogen agonist and antagonist properties ***(Shams-Eldeen et al., 2018).***

Clomiphene has been used to induce ovulation in patients suffering from chronic oligo-anovulation and ovulatory dysfunction. Clomiphene citrate is administered for five days beginning on any menstrual cycle day from 2 to 5, starting with 50 mg/day and increasing to 150 mg/day if anovulatory. If ovulation cannot be achieved at doses of 150 mg/day, the patient is deemed to have clomiphene citrate resistance (CCR). If pregnancy cannot be achieved after six ovulatory cycles, then the patient is deemed a clomiphene citrate failure (CCF) ***(Palomba et al., 2009).***

Studies with clomiphene citrate have shown an ovulation rate of 60–85% and a pregnancy rate of 30–50% after six ovulatory cycles, with an increased risk of multiple pregnancies (5–7%) ***(Kafy et al., 2007).***

Mechanism of Action

Clomiphene citrate, a competitive antagonist of 17 β estradiol competes with endogenous estrogen for nuclear estrogen receptors at sites throughout the body. However, unlike estrogen, clomiphene binds to nuclear estrogen receptors for an extended interval of time and thereby depletes receptor concentrations by interfering with receptor cycling ***(Gupta et al., 2014).***

Reduced negative estrogen feedback triggers normal compensatory mechanisms that alter the pattern of gonadotropin-releasing hormone secretion and stimulate increased pituitary gonadotropin release which, in turn, drives ovarian follicular development ***(Kerin et al., 1985).*** At the pituitary level, clomiphene might also increase the sensitivity of gonadotrophs to GnRH ***(Hsueh and Erickson, 1978).***

In anovulatory women with WHO Type II anovulation, clomiphene has been reported to induce ovulation in 60–85% of patients and achieve a pregnancy rate of 15–50% per woman ***(Brown et al., 2009).***

Anti-estrogenic effects of clomiphene on the endometrium are likely to be one of the causes of suboptimal pregnancy rates in spite of good ovulation rates. In addition to desirable central actions, clomiphene can exert less desirable anti-estrogenic effects at peripheral sites in the reproductive system. Estrogen plays a critical role in the formation of endometrium in natural cycles. Apart from its major role in proliferative phase, it also primes the endometrium for the luteal phase by the further proliferation of the basal cell layer and the induction of P- receptors, thereby ensuring the capacity of the endometrium to become secretory ***(Katzenellenbogen, 1980).*** There are studies that have shown that adverse effects of CC on endometrium can be prevented by administering estrogen together with or after clomiphene ***(Swasti and Kaul, 2005; Kruger et al., 2005).***

Hence to counteract anti-estrogenic effects of clomiphene on endometrium, estrogen supplementation was initiated in the proliferative phase in clomiphene citrate stimulated cycles in the present study.

Structural and molecular changes occur in the endometrium in the secretory phase during the window of implantation (WOI). Endocrine, paracrine, and autocrine factors are involved between the maternal tissue and the implanting blastocyst in a “crosstalk” that performs with

limited efficiency in humans. Implantation requires the synchronous development of a competent blastocyst and an endometrium able to respond to the signals from the blastocyst ***(Nardo et al., 2006).***

The endometrium, with its orchestrated series of changes in preparation for implantation, will be destroyed leading to menstruation if the blastocyst fails to implant. Endometrial receptivity is compounded of morphological features, molecular basis, and genetic evidence ***(Achache et al., 2006; Diedrich et al., 2007).***

Endometrial receptivity accounts for a successful dialogue between progesterone primed endometrium and viable embryo in window of implantation for blastocyst implantation ***(Rehman et al., 2012, 2013).***

Endometrial thickness

Endometrial thickness is One of the strongest predictors of implantation. A number of reports have shown that embryo implantation and clinical pregnancy rates (PRs) are significantly higher in patients with an endometrial thickness >9 mm. Thin endometrium, generally measuring <7 mm, are thought to be less able to support implantation and pregnancy ***(Richter et al., 2007).***