Section A-Research paper

Role of Clomiphene citrate and Sildenafil Citrate in Induction of Ovulation

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Abstract

Background: One definition of infertility that is frequently used by reproductive endocrinologists, the doctors specialized in infertility, to consider a couple eligible for treatment if: A woman under 35 has not conceived after 12 months of contraceptive-free intercourse. Twelve months is the lower reference limit for Time To Pregnancy (TTP) by the World Health Organization. A woman over 35 has not conceived after 6 months of contraceptive-free sexual intercourse. Clomiphene citrate (CC) is the most common and simple method in ovulation induction in women with normogonadotropic anovulatory infertility. Clomiphene is a non-steroidal triphenylethele derivative with both estrogen agonist and antagonist properties .although climiphene citrates acts as antiestrogenic factor in almost of cases .it has a weak estrogenic action which appear when endogenous levels are very low . Clomiphene is cleared through the liver and excreted in the stool, approximately 85% is eliminated with a weak, but traces can remain in the circulation for longer. Sildenafil citrate is a non-steroidal compound that works as anti-oestrogen. It blocks oestrogen receptors at the hypothalamus, releasing it from negative feedback, and augmenting its release of GnRH. Subsequently, pituitary production of gonadotropins is increased resulting in follicular growth, and ovulation.

Keywords: Clomiphene citrate, Sildenafil Citrate, Induction of Ovulation

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Introduction

One definition of infertility that is frequently used by reproductive endocrinologists, the doctors specialized in infertility, to consider a couple eligible for treatment if: A woman under 35 has not conceived after 12 months of contraceptive-free intercourse. Twelve months is the lower reference limit for Time To Pregnancy (TTP) by the World Health Organization. A woman over 35 has not conceived after 6 months of contraceptive-free sexual intercourse. **(1).**

The idea of these time intervals is that woman beyond 35 years old every month counts and if woman advised to wait additional six months to prove the necessity of medical intervention the problem could be worsen . the final result bydefinition is that , failure to conceive in women under 35 isn't regarded with the same urgency as it is in those over 35. Infertility represents about 15%-20% of infertile couples usually these cases need controlled ovarian stimulation with or without assisted reproductive methods. Clomiphene citrate is the traditional used drug for this problem but sometimes pregnancy failed to be achieved with it other options for assistance is gonadotrophin or letrozole. **(1).**

Even with a normal semen analysis, the sperm may still be contributing to infertility. A normal standard semen analysis does not always predict normal sperm/zona pellucida binding or penetration. Another example

of a possible male contribution to infertility is a recently described mutation of a gene encoding a protein called β-Defensin 126, which coats the sperm head and allows for normal sperm transportation through the cervical mucus. The mutation is found in approximately 1 in every 250 men, and when present, measurably lowers fertility. The presence of the mutation cannot be detected by a standard semen analysis, but intrauterine insemination (IUI) or IVF seem to bypass the deleterious effect of the mutation. Unexplained infertility, also called subfertility, is defined as failure to conceive after one year in couples with normal semen samples and no abnormality could be detected during an infertility workup **(2)**.

Epidemiology of unexplained infertility:

A common standard evaluation, as outlined by the Practice Committee of American Society of Reproductive Medicine in 1992 include a semen analysis, an assessment of ovulation, a hysterosalpingogram, and if indicated, a laparoscopy. It is estimated that such an evaluation will fail to identify an abnormality in approximately 15% of couples**.** Prevalence of infertility in Egypt according to a study conducted by the Egyptian Fertility Care Society and sponsored by the World Health Organization, infertility in Egypt affects 12 percent of Egyptian couples **(3).**

Theories on possible causes for unexplained infertility:

Pituitary or follicular dysfunction:

Approximately 5% of women with infertility have elevated levels of follicular stimulating hormone (FSH) in the early follicular phase, which reflects diminished ovarian reserve**.** Furthermore, serum estradiol levels in the follicular phase and the estradiol/progesterone ratio elevated in women with infertility, suggesting altered folliculogenesis. In addition, an absent mid-cycle elevation of prolactin hormone was present in infertile women **.** An impaired luteal phase has been demonstrated in about 30% of women with infertility. An abnormal follicular LH pulse frequency or decreased midfollicular FSH level have been postulated to induce an impaired luteal phase, which may primarily be due to a functional imbalance in the hypothalamus **(4).**

It has been shown that decreased inhibin-B concentrations are associated with increased FSH concentrations, and both may reflect a diminished ovarian reserve, which is sometimes seen in women with infertility. Also, the serum anti-Mullerian hormone level is a marker of ovarian reserve, and has been highly correlated with the number of antral follicles and oocytes retrieved in IVF cycles **(5).**

Gamete dysfunction:

Besides hormonal factors, gamete dysfunction may contribute to infertility. Altered folliculogenesis, impaired oocyte maturation, reduced oocyte quality and defects in gamete interaction have all been suggested**.** Also, sperm dysfunction would impair the ability of spermatozoa to penetrate the cervical mucus, the zona pellucida and the ooplasmic membrane. An insufficient acrosome reaction could cause fertilization failure, which is sometimes seen in IVF**.** Total failure of fertilization is reported in 5–30% of the cycles of women undergoing conventional IVF; this is more frequent than among other infertility subgroups **(6).**

Altered endometrial function:

Another possible explanation for infertility is altered endometrial function. A subacute inflammation may be present, as differences in endometrial leukocyte populations have been observed between fertile.

Low endometrial progesterone receptor concentrations, inadequate estrogenic induction of progesterone receptors, decreased inhibin levels and suboptimal expression of integrins or pinopode formation in the endometrium have also been reported in women with infertility**.** Aberrant patterns of integrin expression, e.g. absence of the avb3 subunit in the window of implantation despite normal histological maturation of the endometrium, have been associated with infertility **(7).**

Altered uterine or spiral artery blood flow:

Abnormal uterine artery blood flow might be associated with infertility. Increased uterine artery impedance, absent end-diastolic flow or an abnormal flow in spiral arteries in the midluteal phase have been suggested to impair the implantation process among these women**.** The mean uterine artery pulsatility index (PI) is normal among women with infertility, in both the pre- and the post-ovulatory periods. However, 15% of women with infertility in their study had a uterine artery PI over 2.8, which is higher than among women with no history of infertility. **(8).**

It was shown that the uterine artery resistance index (RI) on the day of human chorionic gonadotropin (hCG) administration in the IVF cycle was significantly higher in this patient group as compared with women with tubal infertility**.** Poor endometrial blood flow has in some studies predicted poor implantation rate**.** Impedance (resistance) to blood flow in the uterine or spiral artery did not differ in women conceiving with IVF–embryo transfer (ET). Neither did they find any difference in spiral artery impedance when women with unexplained and tubal infertility were compared, and the uterine impedance was even lower on day 5 of the IVF cycle among women with infertility **(8).**

Immunological factors:

Many types of antibody have been suggested as possible causes for infertility. Antiovarian antibodies are frequent (33%) in some reports among women with infertility, as are elevated anti-spermatozoal and anti-cardiolipin antibodies; the antibody levels have been shown to correlate with uterine artery resistance Moreover, inadequate maternal immunosuppression might cause embryo rejection has been suspected**.** The prevalence of celiac disease is also higher among women with infertility compared with both the general population and other infertile women **(9).**

Other factors:

Infertility may also be functionally linked to endometriosis, since many women with infertility later develop endometriosis. Furthermore, occupational exposure to noise, chemicals, radiation, mercury and cadmium may be linked to infertility. Women's anxiety and stress levels may also lower the chances of conception **(8).**

Diagnostic criteria of unexplained infertility:

Unexplained infertility is diagnosed when all of the standard elements of infertility evaluation yield normal results. At a minimum, the diagnosis of unexplained infertility implies a normal semen analysis, objective evidence of ovulation, a normal uterine cavity, and bilateral tubal patency. The necessity for diagnostic laparoscopy in the evaluation of couples with unexplained infertility has been controversial **(10).**

Evaluation of the male partner:

An infertility evaluation should be performed if a couple has not achieved conception after one year of unprotected intercourse. An evaluation should be performed earlier if male or female infertility risk factors exist and if the couple questions its fertility potential.The initial screening of the male should include a reproductive history and a physical examination performed by a urologist or a specialist in male fertility and two semen analysis . Additional procedures and testing may be used to elucidate problems discovered during the full evaluation.When couples seek assistance, an initial semen analysis remains the current standard for male evaluation.A diagnosis of male factor infertility is reached in only 40% of the affected males seeking assistance.**(11)**.

Laboratory evaluation:

Semen analysis may be useful in both clinical and research settings, for investigating male fertility status as well as monitoring spermatogenesis during and following male fertility regulation and other interventions. This manual provides updated, standardized, evidence-based procedures and recommendations for laboratory managers, scientists and technicians to follow in examining human semen in a clinical or research setting. Detailed protocols for routine, optional and research tests are elaborated. To discuss the role, reliability and limitations of the semen analysis in the evaluation of fertility with reference to the World Health Organization (WHO) fifth edition guidelines, with semen analysis reference values published in 2010 **PATEL, A. S. et el (12)** discuss the limitations of using a single threshold value to distinguish 'abnormal' and 'normal' parameters.

Impaired semen parameters alone cannot be used to predict fertility as these men still have a chance of being fertile, except when a man has azoospermia, necrospermia or globozoospermia **(12).**

Semen consists of two components: the spermatozoa made by the seminiferous tubules of the testis, and the seminal fluid produced by the accessory glands that nourishes sperm and has a role in interacting with the female reproductive tract to influence fertility **(13).**

These components are reflected in the semen analysis by the sperm count, which reflects the number of spermatozoa in the semen sample; and the volume of the semen, which reflects the amount of seminal fluid produced **(14).**

Sperm motility is defined as the percentage of sperm that show signs of movement, whilst the sperm morphology is the percentage of sperm that appears to have a normal cellular structure. Sperm vitality is defined as the percentage of sperm that are viable in the sample.

A semen sample is collected by masturbation after an abstinence period of 2–7 days, preferably near the laboratory to limit the time between collection and analysis. The physical characteristics of the semen sample, such as the volume, pH, colour, liquefaction and viscosity is measured, and the sample is then evaluated under a microscope to determine the motility, vitality, concentration, and morphology **(14).**

The values obtained are compared to the reference values determined by the WHO manual.

Semen analysis is the most important laboratory investigation for men when assessing the infertile couple. Advances in in vitro fertilisation (IVF) techniques, particularly intracytoplasmic sperm injection (ICSI) involving the direct injection of a single spermatozoon into an egg, have not diminished the role of semen analysis in modern reproductive practice. Semen analysis is the most basic laboratory investigation undertaken and is descriptive in terms of semen volume, appearance, viscosity, sperm concentration, sperm motility and morphology. Since the results are used by clinicians to choose appropriate treatment options, a reliable service is imperative. It is crucial that the laboratory is experienced in the performance of semen analyses to ensure an accurate result **(15)**.

To ensure a quality semen analysis service, laboratories must participate in internal and external quality assurance activities, incorporate rigorous training protocols for technical staff and use reliable procedures.The World Health Organization laboratory manual for the examination of human semen and sperm cervical mucous interaction, clearly describes the variables that need to be assessed and the methods of analysis and quality assurance to be used **(15)**.

Sperm density is defined as the number of sperm per milliliter of ejaculate. A normal germinal epithelium produces 100 to 300 million sperm per day. Approximately 3 months are required for the production and maturation of sperm within the seminiferous tubules, and another 3 to 10 days are needed for transport through the male reproductive tract **(16)**.

Advanced semen testing:

Following the initial evaluation and semen analysis, advanced testing is indicated to identify defects potentially contributing further to male factor infertility.These include tests for abnormalities of both (a) seminal fluid and (b) sperm function.The decision about which tests are required depends on the history, physical examination, and initial semen analysis).

Evaluation of the female partner:

ovulatory dysfunction:

slight ovulatory dysfunction has been added as an etiology of infertility. A history of regular menstrual cycles is an indication that ovulation is most likely happened .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. The luteinized unruptured follicle syndrome (LUFS) has been suggested to be a cause of infertility in couples in whom the hormonal evaluation is normal, yet conception does not happen. Although this condition arises sporadically with some frequency, no evidence suggests that it occurs consistently in certain women. measurement of midluteal serum progesterone levels is probably the most cost-effective compromise indicator that ovulation has occurred using hormonal methods. Because progesterone is released in a pulsatile manner, more than one assay may be needed to determine serum levels accurately. It diagnosed as having luteal phase defect and the incidence may be higher (approximately 5%) in women with a history of recurrent abortions **(10).**

The diagnosis of LPD should be considered in women with normal cycles and unexplained infertility. However, there were controversies concerning the issues of diagnosis. An important issue is the interpretation of the endometrial biopsies which is variable from one pathologist to another. A further problem is the 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%. These figures suggest that LPD occurs by chance alone **(10).**

Table (1) Summary of the Methods of evaluation of ovulation (17).

Test	**Criteria**	**Advantages**	**Disadvantages**
Menstrual History	Regular, predictable cycles at 25-35 day interval. Consistent flow characteristics and premenstrual moliminal.	No cost. noninvasive.	Subjective unreliable in infertile women
Natural family planning methods	Cyclic changes in cervical mucus characteristics.	No cost . Noninvasive	Subjective unreliable. Unacceptable to many women.
Basal body temperature	Biphasic pattern	Low cost Noninvasive. Defines approximate time of ovulation, Follicular and luteal phase duration.	Many become tedious over time. Interpretation frequently uncertain. Defines approximate time of ovulation only after interval of highest fertility has passed
Serum progesterone concentration	Luteal phase concentration >3ng/ml	Modest cost. Minimally invasive. Simple and objective. Highly accurate if properly timed.	Correct interpretation requires proper timing. Cannot define time of ovulation.
Monitoring urine LH Excretion.	Detection of LH surge.	Modest cost. Relatively simple, Objective, and reliable. Accurately defines the interval of highest fertility in advance of ovulation. Accurately defines follicular and luteal phase duration.	May become costly and tedious over time. Self testing must be performed according to specific instructions. May yield false negative of equivocal results.
Endometrial biopsy	Secretory histology	Objective Highly accurate if properly timed. Specifically confirms or excludes diagnoses of endometrial hyperplasia or chronic endometrilis.	Moderately high cost. Invasive may be associated with significant discomfort. Cannot define time of ovulation. Associated risk greater than with other methods.
Serial transvaginal ultrasound examinations.	Observation of progressive Preovulatory follicular growth and subsequent follicle collapse, increased internal echo density and increased volume of cul-de sac fluid.	Define the size and number of antral follicles. Accurately defines time of ovulation during interval of highest fertility. .Accurately defines follicular and luteral phase duration. Best evidence the ovulation actually occurs.	High cost Moderately invasive Requires frequent office visits. Requires a highly trained and experienced examiner.

Assesment of ovarian, tubal and peritoneal, factors:

1*HSG

Hysterosalpingography is an investigative modality used in the evaluation of the uterine cavity, fallopian tubes, and adjacent peritoneal cavity following the injection of contrast material through the cervical canal.Hysterosalpingography remains the most common method of ascertaining tubal patency in our environment and perhaps the most common form of uterine instrumentation in infertile women**(16).**

Apart from hysterosalpingography, investigative modalities are transvaginal ultrasound scan, hysteroscopy, sonohysterosalpingography, laparoscopy and dye test, and magnetic resonance hysterosalpingogram.

It was found that HSG interpreted as normal in women with unexplained infertility was confirmed at surgery in 96% of cases in comparison with 63.1% of patients with HSG interpreted as suspicious. Swart and colleagues performed a meta-analysis of 20 previously published studies comparing the accuracy of diagnosis of tubal patency or peritubal adhesions by HSG in comparison with laparoscopy. Point estimates for tubal patency of 0.65 and 0.83 for sensitivity and specificity, respectively, were calculated.This means that although tubal disease is very likely in the presence of an abnormal HSG, tubal patency on HSG does not rule out pathology. **(17)**.

The management of miscarriage has radically changed over the past 20 years. The emphasis on urgent surgical slight ovulatory dysfunction has been added as an etiology of infertility. A history of regular menstrual cycles is an indication that ovulation is most likely happened .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. The luteinized unruptured follicle syndrome (LUFS) has been suggested to be a cause of infertility in couples in whom the hormonal evaluation is normal, yet conception does not happen. Although this condition arises sporadically with some frequency, no evidence suggests that it occurs consistently in certain women. measurement of midluteal serum progesterone levels is probably the most cost-effective compromise indicator that ovulation has occurred using hormonal methods. Because progesterone is released in a pulsatile manner, more than one assay may be needed to determine serum levels accurately. It diagnosed as having luteal phase defect and the incidence may be higher (approximately 5%) in women with a history of recurrent abortions **(10).**

The diagnosis of LPD should be considered in women with normal cycles and unexplained infertility. However, there were controversies concerning the issues of diagnosis. An important issue is the interpretation of the endometrial biopsies which is variable from one pathologist to another. A further problem is the 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%. These figures suggest that LPD occurs by chance alone **(10).**

Clomiphene citrate (CC) is the most common and simple method in ovulation induction in women with normogonadotropic anovulatory infertility. It was first synthesized in 1956, introduced for clinical trials in 1960, and approved for clinical use in the United States in 1967 **(18)** .

Clomiphene is a non-steroidal triphenylethele derivative with both estrogen agonist and antagonist properties .although climiphene citrates acts as antiestrogenic factor in almost of cases .it has a weak estrogenic action which appear when endogenous levels are very low . Clomiphene is cleared through the liver and excreted in the stool, approximately 85% is eliminated with aweak, but traces can remain in the circulation for longer **(19).**

Clomiphene is aracemic mixture of two different stereisomers, enclomiphene (cis-clomiphene) and zuclomiphene (trans-clomiphene). Enclomiphene is the most potent isomer and the one responsible for its ovulation induing actions **(10).**

Enclomiphene have a relatively shorter half life so the serum concentrations rise and fall quickly during and after treatment . otherwise zuclomiphene have a longer half life so serum levels still detectable for weeks after a single dose and may even gradually accumulated over aseries of cycles , but there is no evidence that residual zuclomiphene has any important clinical effects or consequences **(19)**.

Mechanism of action:

Unlike estrogen, clomiphene remains bound for an extended interval of time and ultimately depletes estrogen receptor concentrations by interfering with receptor recycling. At the hypothalamic level, estrogen receptor depletion prevents accurate interpertaion of circulating estrogen levels; circulating estrogen levels are perceived as lower than they truly are. Reduced estrogen negative feedback triggers normal compensatory mechanisms that later the pattern of Gonadotropin-Releasing Hormone (GnRH) secretion and stimulate increases pituitary gonadotropin release, which in turn drives ovarian follicular development **(10).**

When administered to already ovulatory women clomiphene increases GnRH pluse frequency, but in anovulatory women with polycysic ovary syndrome in whom the GnRH pluse frequency is already abnormally high, clomiphene increases pluse amplitude and not frequency. Serum levels of both FSH and LH rise during clomiphene treatment and fall again promptly after completion of the typical 5-day course of therapy **(20)**.

In successful treatment cycles, one or more follicles emerge and grow to maturity. In parallel, serum estrogen levels rise progressively, unltimately triggering an LH surge and ovulation. Considering its hypothalamic site of action, it is not surprising that clomiphene is typically ineffective in women with hypogonadotropic hypogonadism **(10).**

Protocol for clomiphene citrate use (dosage and administration)

The traditional dose of clomiphene citrate is for 5 days during follicular phase . starting dose is 50 mg daily with gradual increase to 100 mg 150 mg 200mg daily during consecutive cycles, , in cases of no response. Doses higher than 200mg are in general of no additional benefit whereas the adverse effects, in particular on cervical mucus and the endometrium, are increased **(21).**

Ovulation and conception rates and pregnancy outcomes are similar when treatment starts any where between cycle day 2 and 5. A course of six ovulatory cycles is usually sufficient to show whether pregnancy will be achieved using CC before moving to a more complex treatment. Although CC will restore ovulation in approximately 80% of patients, it will result in pregnancy in only about 35%-40%. However, 20%-25% anovulatory women will not respond at all and are considered to be CC resistant**.** obese, insulin resistant and hyperandrogenic patients is more likely to have ovulation induction with climiphene citrate . Women with high basal LH levels are also less likely to respond to CC treatment **(22).**

an insulin- sensitizing agent like metformin or rosiglitazone may help in promoting spontaneous ovulation or in improving ovulation induction with CC . Alternative treatment for CC failures is the use FSH injections, although care has to be taken to prevent ovarian hyperstimulation especially in PCOS patients in whom multiple follicles are poised to stimulated. Another alternative to CC involves the use of aromatase inhibitors of ovulation induction **(23)**.

Side effects of clomiphene citrate:

Clomiphene citrate for ovulation induction in anovulatory women is considered to be relatively safe because steroid negative feedback remains intact. The oral route of administration and low costs represent additional advantages of his preparation. In addition to its desirable central action of stimulation a transient increase in gonadotropin secretion, CC may have other potentially detrimental effects on peripheral reproductive functions. In vitro studies have revealed inhibition of human granulose or luteal cell steroidogenesis. However, in the context of higher E_2 levels as a result of dominant follicle growth, this is probably not of clinical importance **(21).**

Antiestrogenic effects at the uterine level (cervical mucus production and endometrial receptivity) are believed to underlie the observed discrepancy between achieved ovulation and pregnancy rates. CC is be licensed for just 6 months of use in some countries due to The supposed increased risk of ovarian cancer reported to be associated with the use of CC for more than 12 months **(24).**

Hot flushes may occur in up to 10 % of patients using cc , side effects are rare. Nausea, vomiting, mild skin reactions, breast tenderness, dizziness, and reversible hair loss have been reported, but less than 2% of women are affected. The mydriatic action of CC may cause reversible blurred vision in a similar number of women . Overall side effects are CC dose-related and are completely reversible once medication is stopped. Sildenafil citrate is a a 5-phosphodiesterase inhibitor widely used for male erectile dysfunction, Sildenafil citrate acts

by inhibiting cGMP-specific phosphodiesterase type 5, an enzyme that promotes degradation of cGMP, which regulates blood flow in the penis. Since becoming available in 1998, sildenafil has been the prime treatment for erectile dysfunction; its primary competitors on the market are tadalafil and vardenafil. Phase I clinical trials under the direction of *Ian Osterloh* suggested that the drug had little effect on angina, but that it could induce marked penile erections. *Pfizer* therefore decided to market it for erectile dysfunction, rather than for angina. The drug was patented *in 1996*, approved for use in erectile dysfunction by *the United States Food and Drug Administration on 1998*, becoming the first oral treatment approved to treat erectile dysfunction *in the United States*, and offered for sale *in the United States* later that year **(25)**.

Dosage:

The dose of sildenafil for erectile dysfunction is 25 mg to 100 mg taken not more than once per day between 30 minutes and 4 hours prior to sexual intercourse **(26)**.

Adverse effects:

The most common adverse effects of sildenafil use included headache, flushing, dyspepsia, nasal congestion and impaired vision, including photophobia and blurred vision. Some sildenafil users have complained of seeing everything tinted blue (cyanopsia). Some complained of blurriness and loss of peripheral vision **(26)**. The US Food and Drug Administration found that sildenafil could lead to vision impairment in rare cases and a number of studies have linked sildenafil use with nonarteritic anterior ischemic optic neuropathy **(27)**.

Rare but serious adverse effects found through postmarketing surveillance include priapism, severe hypotension, myocardial infarction (heart attack), ventricular arrhythmias, stroke, increased intraocular pressure, and sudden hearing loss **(26)**.

As a result of these postmarketing reports, in 2007, the FDA announced that the labeling for all PDE_5 inhibitors, including sildenafil, required a more prominent warning of the potential risk of sudden hearing loss **(28)**.

Contraindications in newborns:

Contraindications include:

- When taking nitric oxide donors, organic nitrites and nitrates, such as glyceryl trinitrate (nitroglycerin), sodium nitroprusside and amyl nitrite ("poppers").
- Severe hepatic impairment (decreased liver function).
- Severe impairment in renal function.
- Hypotension (low blood pressure).
- Recent stroke or heart attack.
- Hereditary degenerative retinal disorders (including genetic disorders of retinal phosphodiesterases).

(28)

Role of sildenafil in ovulation induction:

CC is a non-steroidal compound that works as anti-oestrogen. It blocks oestrogen receptors at the hypothalamus, releasing it from negative feedback, and augmenting its release of GnRH. Subsequently, pituitary production of gonadotropins is increased resulting in follicular growth, and ovulation.Ovulation rates with CC approach 70- 85% per cycle, whereas cumulative pregnancy rates in a 6-month period is 40-70%. About 15% of anovulatory women do not respond to CC **(29)**.

This is identified as clomiphene citrate resistance. On the other hand, clomiphene citrate failure refers to another subset of women who do not get pregnant despite achieving ovulation after CC treatment. Management of women with clomiphene citrate resistance or failure consists of gonadotropin induction of ovulation or laparoscopic ovarian drilling in PCOS. Ovulation induction with gonadotropins is expensive, requires frequent monitoring, is associated with higher risk of ovarian hyperstimulation syndrome, and multiple pregnancies Similarly, laparoscopic ovarian drilling is associated with potential damage to ovarian reserve, and possible adhesion formation **(29)**.

Therefore, analysis of factors leading to clomiphene citrate failure is necessary to make full use of this inexpensive, relatively safe, and effective method of ovulation induction. An important cause of clomiphene citrate failure is its anti-estrogenic effect on the endometrium. As oestrogen is necessary for endometrial

growth during the follicular phase of the cycle, the antiestrogenic effects of CC impair endometrial growth. This is evident in endometrial biopsies from women on CC, which show impaired epithelial proliferation, decreased number and diameter of glands, with delayed glandular maturation. Sonographically, this poor endometrial development is identifed as thin endometrium. Oestrogen mediated growth of the endometrium is dependent on the blood flow to the endometrium. It is then logical to think of vasodilator therapies as potential solutions to improve endometrial blood flow and thickness in infertile women with clomiphene citrate failure **(30)**.

Sildenafil is a type 5-phosphodiestrase inhibitor that augments vasodilator effect of nitric oxide on vascular smooth muscles by preventing the degradation of cGMP. Sildenafil citrate could lead to an improvement in uterine blood flow and, in conjunction with oestrogen, it leads to the oestrogen-induced proliferation of the endometrium.Itwas addressed That the use of sildenafil to increase endometrial thickness in CC stimulated cycles. Sildenafil was given as vaginal suppositories and in multiple doses per day. In addition, it was not clear whether the studied population had persistently thin endometrium with prior cycles of CC induction.

In addition, for women with prior failed IVF/ICSI cycles associated with thin endometrium, using sildenafil vaginal suppositories during their subsequent cycles expanded their endometrial growth, enhanced endometrial blood flow, and improved their pregnancy rates **(30)**.It was addressed the relation between endometrial thickness and pregnancy outcome in CC induction cycles. Thin endometrium was associated with non-conception, while **Asante et al. (31)** found that preovulatory endometrial thickness was of limited predictive ability for pregnancy.

The reason for these contradicting results may be because of different stimulation protocols (CC alone, CC+ HMG, or IVF/ICSI). different cut-off values to define thin endometrium (6, 7, or 8 mm), and variable timing of endometrial thickness measurement (day of HCG administration, or fixed cycle day 10 or 12 **(31).** Despite this debate, endometrial thickness below 7 mm is generally considered suboptimal for pregnancy. The mechanism by which clomiphene citrate causes thinning of the endometrium is unknown. Working as an antagonist at the endometrial oestrogen receptor level is a major cause. In addition, diminished uterine and endometrial blood flow in clomiphene citrate stimulated cycles might also play a pathogenic role **(30)**.

Sakhavar et al. (32) could not find significant difference in uterine artery pulsatility or resistive indices among unexplained infertility patients treated with clomiphene citrate, lotrezole, or control women. This is taken as a basis for an argument that diminished endometrial blood flow is not a cause for impaired endometrial growth in clomiphene citrate treatment. However, they recruited normo-ovulatory women with unexplained infertility. In addition, there was no assessment of endometrial thickness in this study. It was shown that uterine perfusion in clomiphene citrate stimulated cycles is significantly lower than natural cycles on the day of ovulation. **Miwa et al. (33)** reported significantly higher resistive index in uterine radial arteries in women with thin endometrium than in women with normal thickness endometrium during different phases of the menstrual cycle.

It was detected improvement in endometrial thickness, and uterine radial artery RI with the use of different vasodilator agents (vitamin E, L-arginine, and vaginal sildenafil acetate) in patients with thin endometrium (< 8 mm), and high radial artery RI. It was noted that there is high uterine vascular resistance and impaired endometrial blood flow in women with clomiphene citrate-associated thin endometrium, and reversing this effect might help to improve endometrial thickness.

Vaginal administration of the sildenafil would achieve high concentration at the endometrium, meanwhile avoiding the well-known systemic side effects of sildenafil such as headache, hypotension, and flushing **(31)**. **Das et al. (34)** have used vaginal sildenafil in the form of suppositories that were prepared from oral tablets. However, there is no registered vaginal suppository form of sildenafil citrate in the market.

Fahmy et al. (35) studied the effect of oral sildenafil citrate 25 mg on pregnancy rate as primary outcome and the endometrial thickness and number of follicles as secondary outcome in women undergoing induction of ovulation. They concluded that sildenafil citrate increased pregnancy rate in females undergoing induction of ovulation by clomiphene citrate 50 mg which may be attributed to the increase in endometrial thickness and number of follicles.

Sildenafil with clomiphene citrate in unexplained infertility:

Sildenafil citrate could lead to an improvement in uterine blood flow and, in conjunction with oestrogen, it leads to the oestrogen-induced proliferation of the endometrium. It was addressed that the use of sildenafil to increase endometrial thickness in CC stimulated cycles. Sildenafil was given as vaginal suppositories and in multiple doses per day**.(34)**

Fetih et al. (36) evaluated the use of a new formulation of topical sildenafil (sildenafil vaginal gel) to increase endometrial thickness and uterine blood flow in women with clomiphene citrate failure due to thin endometrium. They have shown that sildenafil vaginal gel significantly increased endometrial thickness, uterine blood flow and may increase pregnancy rate in anovulatory patients with clomiphene citrate failure due to thin endometrium. The mucoadhesive vaginal gel formulation of sildenafil allowed shorter duration of drug application, and less frequent administration per day.

Hamid et al. (37) evaluated the endometrial thickness, pattern, and endometrial flow, in patients taking clomiphene citrate plus sildenafil tablets introduced vaginally as compared to patients who had no pregnancy on clomiphene citrate alone in unexplained infertility. They concluded that the use of sildenafil as an enhancer of endometrial vascularity helps in improving pregnancy rates in patients with unexplained infertility treated with clomiphene, and counteracts the poor effect of clomiphene on endometrial receptivity

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