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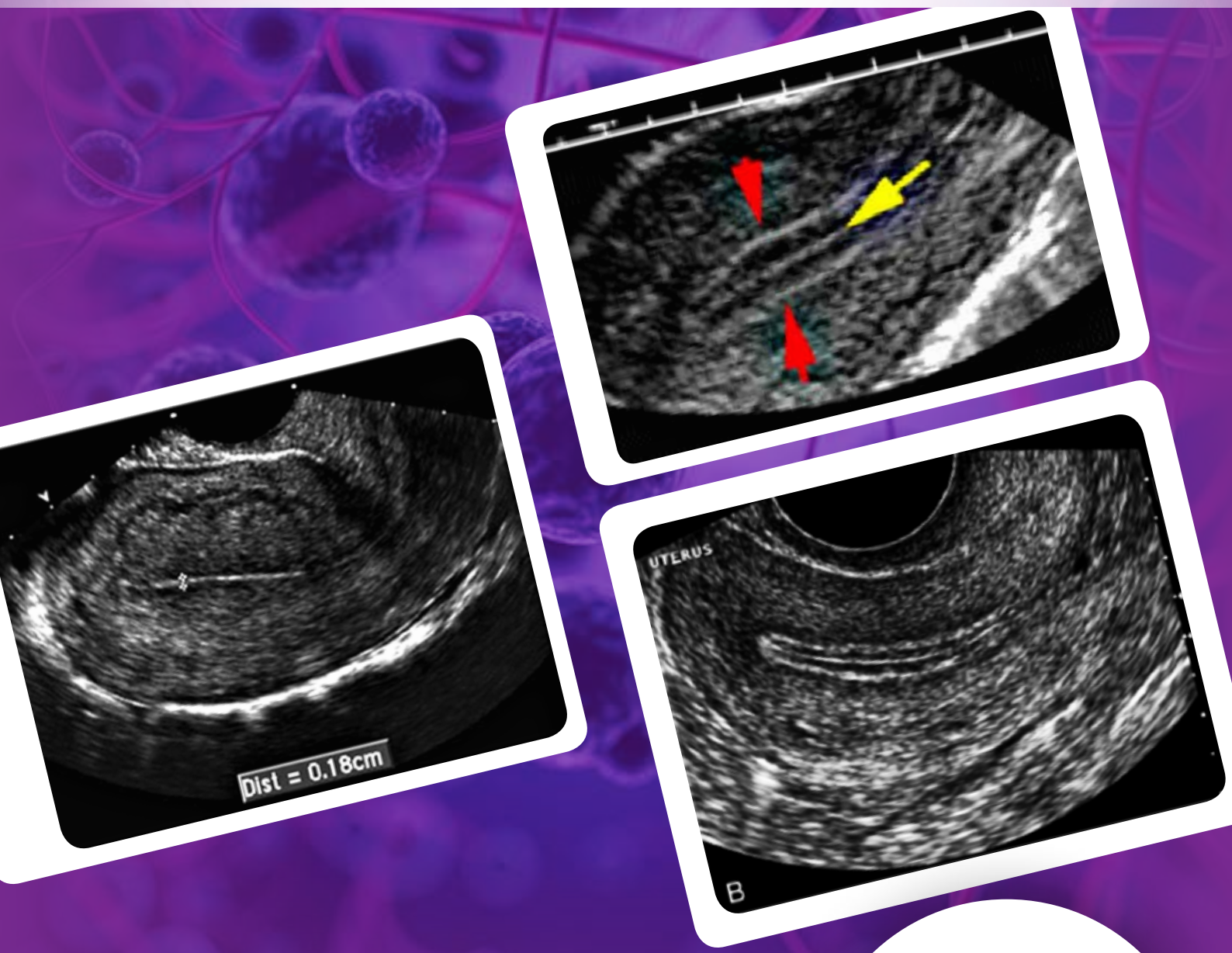
Thin endometrium in Assisted Reproductive Technology



Editor :
Prof (Dr) Pankaj Talwar

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Thin endometrium in ART

DR SOHANI VERMA

PRESIDENT-IFS

It is a great privilege and pleasure to write this message for the fifth E-bulletin of IFS-ARTexT on "Thin Endometrium". Endometrium plays a more active role than previously thought in determining whether the embryo will implant or not.

Thin endometrium is a common challenge in ART which need to be simplified. With this new edition of the bulletin, we have tried to answer questions about the etiologies, symptoms and currently available strategies to improve the endometrium lining in women with thin endometrium.

On behalf on Indian Fertility Society, I sincerely thank "Cadila Healthcare Ltd." for participating with us in this academic endeavour.

DR. K. D. NAYAR

SECRETARY GENERAL IFS

This is an honour for me to write best wishes message for this E-bulletin of IFS-ARTexT. We have always believed in spreading awareness about the common issues in ART and tried to gather and present the evidence that will undoubtedly help both the clinicians and the patients. We intend to cover common day-to-day challenges in clinical ART and thus bring out this E-bulletin named ARTexT. The aim would be to simplify the complex issues in clinical ART and present before you in concise manner. I am sure that you would appreciate and learn from this academic pursuit of the IFS. In this issue we would be covering "Thin Endometrium" which is still an enigma. This manual may help you find the required answers for the queries related to this topic.

Prof (Dr) Pankaj Talwar
Joint Secretary-IFS
Chief Editor ARText

At the very onset, the editorial team would like to thank all of you for reading this E-bulletin of ARTexT. It was my dream to create a bulletin on the lines of NEXUS, which would cover all essential measures of a burning issue in clinical ART. We intend to cover every topic in great detail touching on basic sciences and advanced management and the controversies. The bulletin has been named ARTexT- which means amalgamating different clinical conditions in ART and Reviewing the Text.

The present edition is focused on "Thin Endometrium" which occurs when the uterine lining is less than 7 mm. Lining is considered satisfactory for a successful pregnancy when its thickness is 8 mm as it nourishes the foetus. Number of factors contribute to thin endometrium, few of them like excessive use of Clomiphene, pelvic surgeries, amenorrhea, fibroids and pelvic inflammatory diseases. Endometrium thickness is used as a significant marker of receptivity and as a prognostic factor in embryo transfer.

Several methods and drug protocols have been tried like Vitamin E, estrogen release, Sildenafil citrate, G-CSF, platelet rich plasma and stem cell therapy. We have tried to brief the latest scientific studies and different regimes for the beginners and the experienced alike and hope the bulletin would help you to improve your results.

The bulletin is penned in two parts. Part 1 deals with the basics of thin endometrium. Part 2 deals with the frequently asked questions debatable issues concerning ART and the disease .

I am sure this bulletin will immensely benefit you all. Team ARTexT sincerely hopes to bring out such teaching material for you regularly. It would not only help to disseminate scientific and ethical content but also constantly update everyone with new researches and developments across the world.

We would also like to place an record our truthful thanks to Cadila Healthcare Ltd. For helping us in this publication and off course I promise that there is no conflict of interest at any level.

Wish you a happy reading and yes don't forget to file this issue.

I would formally like to thank Dr. Rashmi Sharma and Dr. Ranu Dadu from Origyn Fertility and IVF, New Delhi, who have worked un-relentlessly toward bringing out this issue from conception to end.

Jaihind

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Guest Editor

Thin endometrium is a very frustating problem for both patients and doctors alike with very little therapeutic modalities available to us . A good endometrium is essential for successful implantation of embryo. Thin endometrium is difficult to treat ,though several modalities have been tried like extended estrogen regimen , low dose aspirin , Tamoxifen, sildenafil citrate , G-CSF , Vit E , pentoxiphylline , Platelet rich plasma and stem cell therapy . The latest scientific evidence available regarding these modalities have been briefed in the article . We hope that the knowledge gained will be helpful in treating patients with thin endometrium .

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Subeditor

It is my privilege to write in this prestigious E-Bulletin of IFS – ARText, on a very important topic of thin endometrium . A healthy uterus with adequate endometrium can give a pregnancy at advanced age also. On the contrary a thin endometrium can be a frustrating cause of recurrent implantation failures even in young patients. Continuous efforts have been made to find a treatment of thin endometrium. This is an article to State the etiology, monitoring and treatment options available for thin endometrium.

Notes

Index

Part -1

I. History

It is difficult to say who first draw the attention towards the clinical problem of thin endometrium , though **Heinrich Fritsch** first described Asherman syndrome in 1894 and this problem was further elaborated by **Israeli** gynaecologist **Joseph Asherman** in 1948.

II. Introduction

The uterine cavity is only a thin cleft and is lined by endometrium. The endometrium, derived from the mucosal lining of the fused müllerian ducts, is essential for reproduction and may be one of the most complex tissues in the human body. It is always changing, responding to the cyclic patterns of estrogen and progesterone of the ovarian menstrual cycle, and to a complex interplay among its own autocrine and paracrine factors.

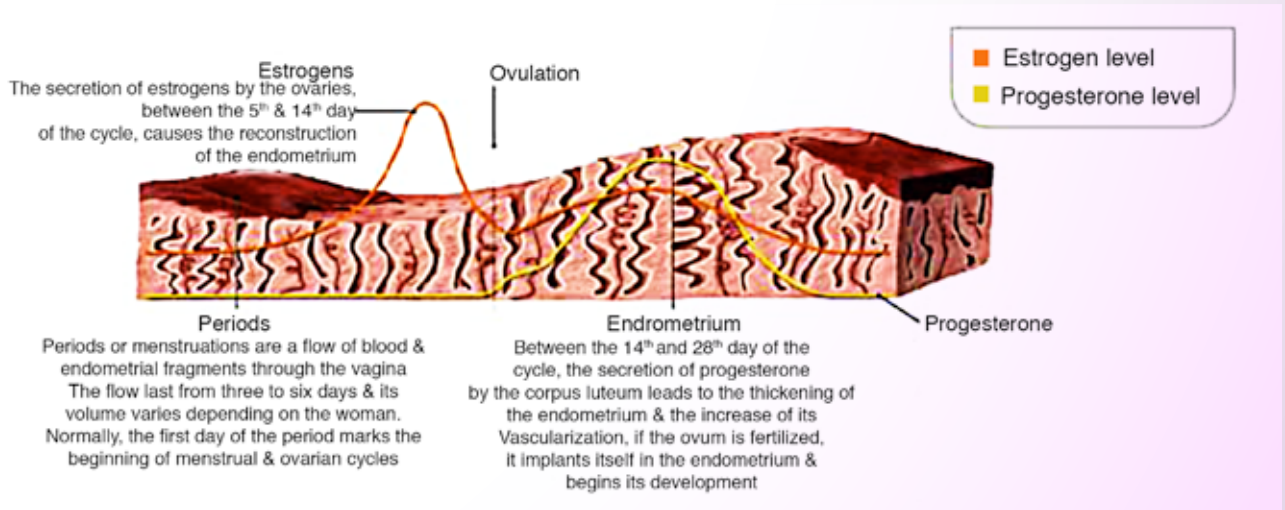
III. Anatomy of endometrium

The endometrium can be divided morphologically into an upper two-thirds **"functionalis"** (stratum compactum and stratum spongiosum) layer and a lower one-third **"basalis"** layer. The purpose of the functionalis layer is to prepare for the implantation of the blastocyst and, therefore, it is the site of proliferation, secretion, and degeneration. The purpose of the basalis layer is to provide the regenerative endometrium following menstrual loss of the functionalis. (**Speroff clinical gynecologic endocrinology 9th edition)**

Before puberty, the endometrial tissue is inactive; it is composed of tubular glands, a dense fibroblastic stroma, and thin blood vessels. The advent of cyclic pituitary and ovarian hormonal activity results in endometrial cyclic morphologic changes involving glands, stroma, and blood vessels that can be identified as characteristic for each day of the cycle.

IV. Cyclic changes in the endometrium

In normal cycles, the menstrual shedding is followed by endometrial proliferation under estrogenic stimulation. The endometrial thickness increases more than 10-fold as a result of active growth of glands, stroma, and blood vessels.

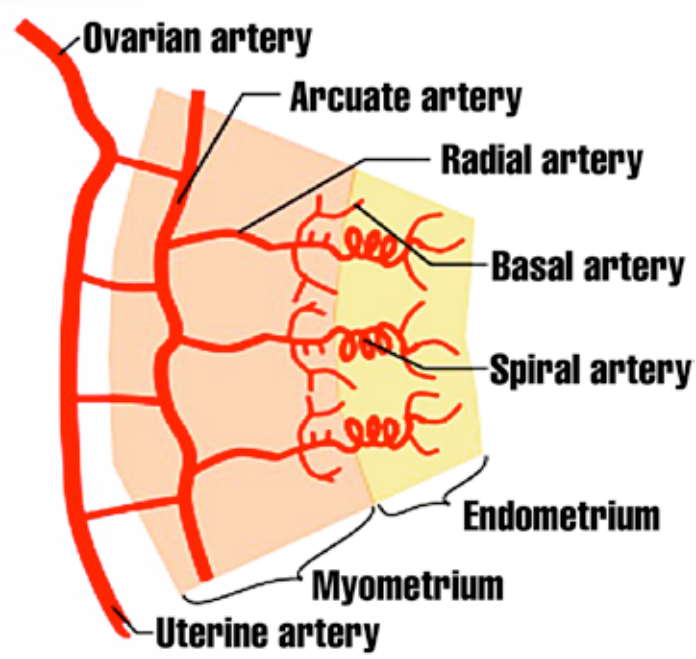


The proliferative phase has a variable length from 10 to 20 days, with an ideal duration of 14 days. During this phase, the endometrial glands grow and become tortuous because of the active proliferation

of epithelial cells. These estrogen-induced changes occur as a response to the binding of the hormone to nuclear receptors. The presence of estrogen receptors in the nuclei of endometrial cells is responsible for the prompt translation of hormonal impulses into structural changes, including, at an ultrastructural level, protein synthesis by free ribosomes and by the rough endoplasmic reticulum and accumulation of intermediate filaments, mitochondria, Golgi apparatus, and lysosomes. The nuclei increase in size, nucleoli become prominent, and chromatin is coarse. The individual cell mass increases as does the nuclear-cytoplasmic ratio. Numerous mitoses are seen throughout the glands and stroma.

After ovulation, the secretion of progesterone inhibits the proliferative activity of the endometrium and induces a complex secretory activity starting with the polarization of glycogen at a subnuclear location followed by its transport via microfilaments to the apical region of the cell. The Golgi apparatus packages the glycogen and various other substances, which become secretory granules and are eventually expelled into the glandular lumen. The secretory changes take place only in an estrogen-primed endometrium.

The secretory activity in the second half of the menstrual cycle is characterized by a diversity of structural changes that are apparent on routine examination of endometrial biopsies, showing a different pattern on every day of the cycle. Dating the endometrium is identifying morphologic changes characteristic for early, middle, and late proliferative endometrium and for each of the 14 days of secretory endometrium **(Noyes et.al. 1973, Ferenczy A. 1994)**.



V. Cyclic changes in the vasculature of endometrium

Perhaps the most significant change in terms of adequacy of the luteal phase is that involving the blood vessels. The thin endometrial arterioles undergo a process of endothelial proliferation, thickening of the wall, and coiling forming the spiral arterioles on the ninth postovulatory day. These arterioles have a critical role in the process of implantation because of the tropism for arterial blood that characterizes trophoblastic tissue. Their coiling increases the surface to be tapped by the implanting trophoblast; therefore, the chances for a normal implantation are reduced if the spiral arterioles are not well developed. The evaluation of endometrial biopsies properly performed a few days before the onset of menstrual shedding is one of the most important diagnostic tools in the work-up of infertility: It offers an insight into the tissue that will play host to the implanting conceptus, assessing its adequacy.

VI. Ultrasound monitoring of endometrium

Different strategies have been developed to evaluate endometrial receptivity, such as the histologic dating of an endometrial biopsy, endometrial cytokines in uterine flushing, the genomic study of a timed endometrial biopsy or more commonly a non-invasive ultrasound examination of the endometrium.

The endometrium is usually best seen on endovaginal scans. By 2D USG the endometrial thickness and pattern can be assessed. Other parameters like endometrial volume and Doppler USG for uterine and endometrial zonal flows can be done by 3 dimensional and 4 dimensional ultrasound.

Endometrial thickness is measured from echogenic border to echogenic border across the endometrial cavity on a sagittal midline image.

Grading of endometrial morphology (Pattern) -

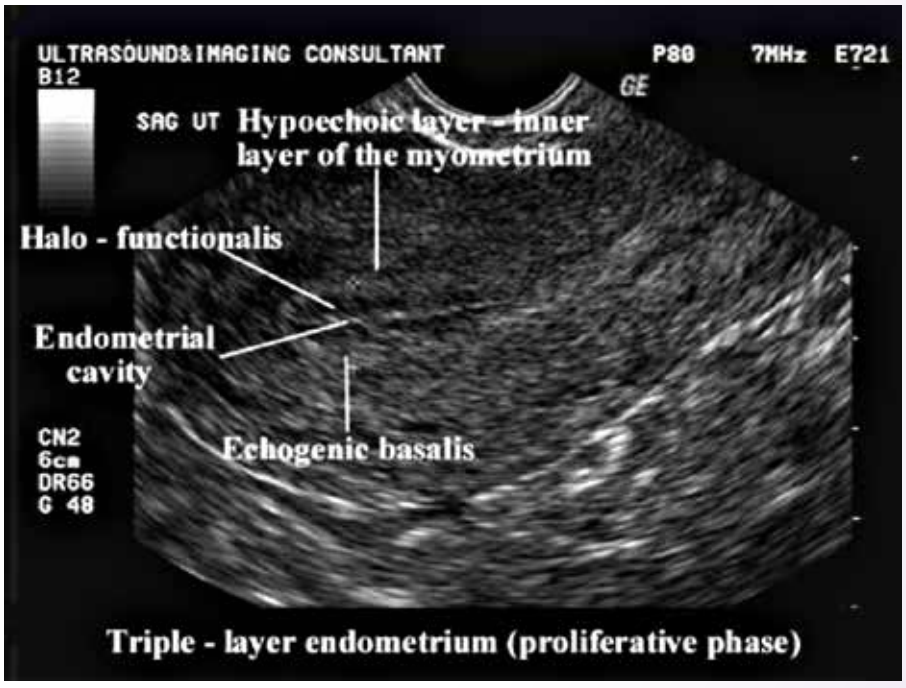
3 grade system by **Gonen and Casper** in 1990.

Type a : Entirely homogeneous, hyperechogenic pattern, without a central echogenic line

Type b : Intermediate iso-echogenic pattern, with the same reflectivity as the surrounding myometrium and a non-prominent or absent central echogenic line

Type c : Multilayered 'triple-line' endometrium consisting of a prominent outer and central hyperechogenic line and inner hypo-echogenic or black region.

Vascularity zones have been defined as:



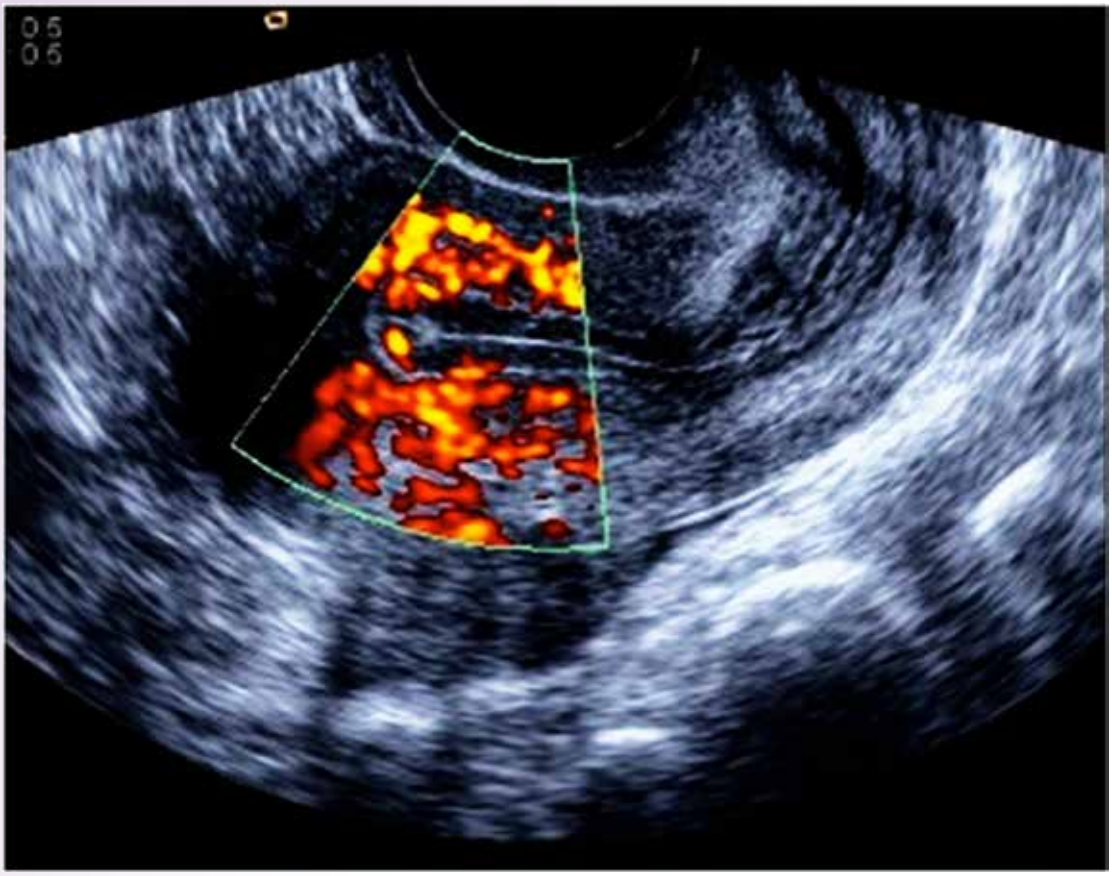
Triple - layer endometrium (proliferative phase)

Zone I - the sub-endometrial zone,

Zone II - the outer hyperechogenic zone and

Zone III - the inner hypoechogenic zone.

This is according to the penetration of blood vessels in endometrial and subendometrial regions .



As **proliferative phase** of the menstrual cycle (days 6–14) begins, the endometrium becomes thicker (5–7 mm) and more echogenic relative to the myometrium, reflecting the development of glands, blood vessels, and stroma. In the **late proliferative (periovulatory) phase,** the endometrium develops a multilayered appearance with an echogenic basal layer and hypoechoic inner functional layer, separated by a thin echogenic median layer arising from the central interface or luminal content. In this stage, the endometrium may measure up to 11 mm in thickness. The layered appearance usually disappears 48 hours after ovulation.

During the **secretory phase,** the endometrium becomes even thicker (7–16 mm) and more echogenic. This increased echogenicity is thought to be related to stromal edema and glands distended with mucus and glycogen.

(Lina Wang et.al. 2010, De Geyter C et.al. ,2000)

Phases of menstruation			
Phases	**Days**	**Thickness of ET**	**Epithelium**
Menstrual phase	**1-5**	**Thin**	**Absent**
Follicular phase	**5-14**	**Intermediate**	**Columnar**
Luteal phase	**15-27**	**Thick**	**Columnar**
Ischaemic phase	**27-28**		**Columnar**

VII. Factors affecting endometrium

3 important factors that affect the growth of the endometrium are as follows

1.Serum Estradiol levels

2. Blood flow to the uterus

3. Health of the Endometrial tissue itself (Previous injuries or Infections)

Woman's age also has an indirect factor on endometrium. (**De Geyter et.al.2000, Ernest Hung et.al. 2006)**

VIII. Where to draw the cut off - How thin is thin endometrium?

There is no definite cut off for thin endometrium. Endometrial thickness has high specificity and sensitivity in predicting pregnancy outcomes. Endometrial pattern independently affect endometrial receptivity and pregnancy outcome .

Various studies have taken as it as < 6mm , < 7mm and sometimes < 8mm. 7mm is most accepted although pregnancies have been reported at very thin endometrium as well.

In a study by **Jing Zhao Et Al** - Implantation rates and clinical pregnancy rates were higher in patients with endometrial thickness greater than 7mm. Patients with multilayered 'triple-line' endometrium consisting of a prominent outer and central hyperechogenic line and inner hypo-echogenic or black region showed higher implantation rates. **(type C)**

IX. Causes of thin endometrium

Cause of thin endometrium is most commonly Iatrogenic or inflammatory -

a. Iatrogenic - Iatrogenic injuries to the endometrial layer can happen during the course of D&C , myomectomy in which endometrium is opened or intracavitory myomectomy , caesarean section in which uterine cavity has been curetted to remove placental bits , polypectomy etc. It is difficult for the endometrium to grow again if the basalis layer is damaged during the course of surgical treatment. **(Sergio Reis Soares et.al. 2008, Mahazan N.et.al. 2016, Miwa H.et.al. 2009, Takasaki et.al. 2010,R Davar 2013)**

b. Inflammatory - In our country tuberculosis of the endometrium is very important and common cause of thin and irreversibly damaged endometrium. Apart from tuberculosis, chronic bacterial infections, sexually transmitted infections and pelvic inflammatory diseases can lead to permanent scarring of endometrium.

c. Other causes

1. Low estrogen levels -

Endometrium is estrogen dependent tissue and if due to any reason estrogen stimulation is not sufficient, the endometrial layer will remain thin as seen in prepubertal girls, postmenopausal women and in cases of hypo gonadotropic hypogonadism.

2. Excessive use of clomiphene -

Clomiphene is an ovulation induction drug with anti-estrogenic effect on the endometrium. Repeated use of clomiphene can lead to estrogen receptor down regulation in the endometrium and persistently thin endometrium for a long duration.

3. Prolonged use of progesterone and combined contraceptive pills -

Oral contraceptives and progestins used for a long period of time usually lead to thinning of endometrial lining.

(Sergio Reis Soares et.al. 2008, Mahazan N.et.al. 2016, I.Miwa H.et.al. 2009 , Takasaki et.al. 2010,)

4. Inadequate blood flow

Uterine blood flow is an important factor controlling endometrial growth and is closely associated with vascular development of the endometrium.

In 2010 Takasaki et. Al. demonstrated that a "thin" endometrium was characterized by high blood flow impedance of RA, poor epithelial growth, decreased VEGF expression, and poor vascular development. It is likely that endometrial growth depends at least partly on angiogenesis and uterine blood flow. Therefore, a thin endometrium may be due to impaired angiogenesis and low uterine blood flow.

High blood flow impedance of radial arteries, which could be a trigger, impairs the growth of the glandular epithelium and results in a decrease in VEGF levels in the endometrium. Low VEGF causes poor vascular development, which in turn further decreases blood flow in the endometrium. The vicious circle leads to a "thin" endometrium that is related to impaired endometrial receptivity. They also suggested that high blood flow impedance of radial arteries at the start of the menstrual cycle can be a useful predictor of a thin endometrium, although the cause of high blood flow impedance of radial arteries in patients with a thin endometrium is unclear.

5.Systemic causes

Certain diseases like hypertension, Diabetes Mellitus, Asthma, Depression, Epilepsy, Substance abuse like smoking are also said to increase the incidence of thin lining.

6. Idiopathic

It is not necessary that thin Endometrium will be secondary to a particular disease. It may vary due to individual uterine architecture as well.

X. Treatments available for Thin Endometrial Lining

Endometrial thickness has long been used as a marker of receptivity of the endometrium and as a prognostic factor in embryo transfers. Improving endometrial growth in patients with a thin endometrium is very difficult.

Traditionally low dose aspirin, vaginal sildenafil, pentoxfylline, tocopherol, and estrogen are administered for building the endometrium. Many a times administration of these agents is ineffective .Many patients fail to achieve the minimum thickness of endometrium.

We will discuss some novel approaches to improve the endometrium in implantation failures in frequently asked questions.

Part -2

Frequently Asked questions (FAQs)

XI. Role of low dose aspirin -

Low dose aspirin increases the uterine blood flow by decreasing the impedance to blood flow measured by decrease in pulsatility index of uterine arteries. **Wada et al. in 1994** studied the use of aspirin treatment for Frozen Embryo cycles in patients with high Doppler pulsatility index (low uterine perfusion). They reported an improvement in the pulsatility index with aspirin treatment, a trend toward an improvement in the pregnancy rate.

To study the effect of Low dose aspirin on endometrial receptivity in patients with with thin endometrium (<8mm), **Louis et. Al.,1997** did a randomized controlled study on 28 patients undergoing donor oocyte ivf and divided into 2 groups. One group received only estrogen and other received low dose aspirin along with estrogen. Although there was no increase in endometrial thickness in the aspirin treated group, but the implantation rates and the clinical pregnancy rates were significantly better in the aspirin treated group.

Similar results were obtained in a study by **Heish et al, 2000** in patients having thin endometrium and undergoing Intrauetrine insemination after low dose aspirin administration.

In **2007 Gelbaya Et al** performed a systematic review and meta-analysis on the role of low dose aspirin on the effect on pregnancy rates in un selected patients undergoing In vitro fertilization and found no significant difference in the clinical pregnancy rates in the groups receiving low dose aspirin. **Cochrane review in 2010 (Glujovsky D et.al.)** found no benefit in adding aspirin for endometrial preparation.

Recent meta-analysis by **Wang et.al. In 2017** included thirteen randomized controlled trials with 3104 participants. There were no significant differences in implantation rate, live birth rate ,miscarriage rate ,fertilization rate and endometrial thickness but it showed that aspirin treatment may slightly improve the clinical pregnancy rate (RR=1.16; 95% CI=1.04-1.28) compared to placebo or no treatment.

Further high quality studies are warranted before a final word can be said about role of ecospirin in improving endometrium.

XII. Role of Pentoxiphylline and tocopherol

Tocopherol (vitamin E) is a potential anti-oxidant and scavenges reactive oxygen species (ROS) at times of oxidative stress. It also has effect on blood vessels. **Takasaki et al. (2010)** demonstrated in a prospective observational study that Vitamin E improves the endometrial thickening through increase in VEGF expression: the study showed an improvement of uterine radial artery-resistance index in 72% (18/25) of patients and of endometrial thickness in 52% of patients (13/25patients). **Cicek N et al.** (2012) conducted a prospective, randomized controlled clinical trial on 103 patients of unexplained infertility undergoing ovulation induction and IUI , found that infertile women treated with Tocopherol had significantly thicker endometrium (mean of 9.6 vs 8.2 mm) but no significant difference in implantation or pregnancy rates .They concluded that Vit E administration may improve the endometrial response in unexplained infertile women via the likely antioxidant and the anticoagulant effects.

Pentoxiphylline (PTX), a methylxanthine derivative, is a vasodilator that enhances red blood cell deformability, inhibits inflammatory reactions and reduces blood viscosity by inhibiting platelet aggregation. It decreases the local production of tumor necrosis factor

Combination of tocopherol and pentoxyphylline has been reported to be useful in radiation induced fibrosis in experimental model and in humans **(Delanian et al., 2003)**

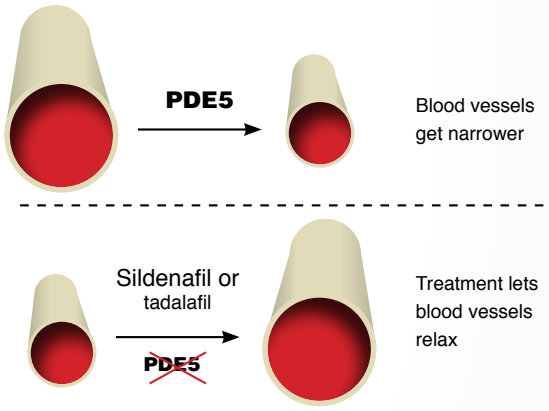
N. Lédée-Bataille et al in 2002 tried combined administration of pentoxiphylline 800mg /day and vit E 1000mg/day in 18 oocyte recipients with refractory thin endometrium(<6mm). In 72% patients (13/18) endometrial thickness improved significantly with pregnancy rate of 33% and live birth rate of 27%. They concluded that combined PTX and vitamin E treatment appears to improve pregnancy rate by improving endometrial thickness.

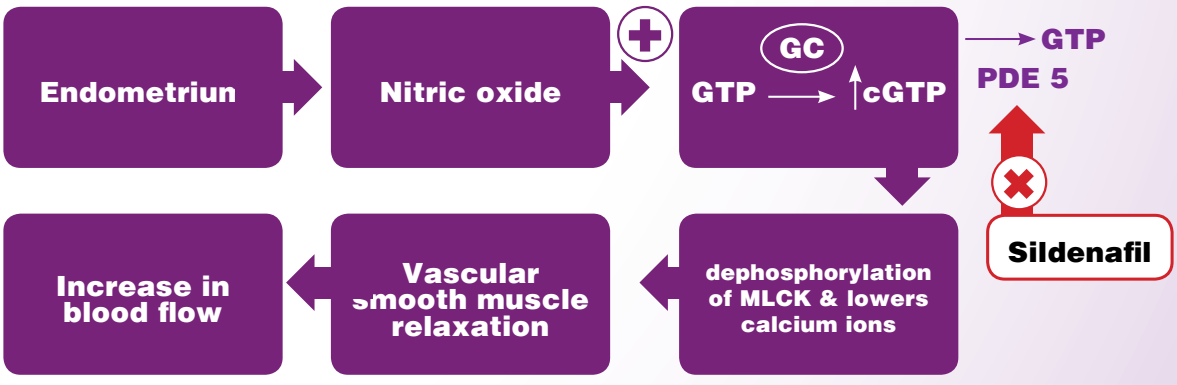
Letur-Konirsch and Delanian (2003) reported Successful pregnancies in 2 out of 3 women with premature ovarian failure and refractory thin endometrium after combined pentoxifylline-tocopherol treatment for 9 months

Acharya et al.(2009) reported on 20 cases of women with thin endometrium undergoing assisted reproduction therapy . They were administered pentoxiphylline and Vit E for approximately 8 months .They reported a significant increase in endometrial thickness at the end of the treatment (4.9 ± 1.5 mm vs. 7.4 ± 0.9 mm, P = 0.001). pregnancy rate was 40 %.

XIII. Role of sildenafil in thin endometrium

Thickness of endometrium depends mainly on uterine artery blood flow. Nitric oxide is a mediator of vascular smooth muscle dilation in many areas of the body. It works by a CGMP-mediated pathway. Sildenafil is a potent and selective inhibitor of cGMP specific phosphodiesterase type 5 (PDE5) that prevents the breakdown of cGMP and potentiates the effect of nitric oxide on vascular smooth muscles. With the use of sildenafil, cGMP levels remain elevated, which leads to vascular relaxation and increased blood flow **(Patil CS et.al. 2005 , Jackson G ,2006)**





GC - Guanylate cyclase; GTP- Guanosine monophosphate; GMP-Guanosine monophosphate;
cGMP - cyclic Guanosine monophosphate; MLCK -Myosin light chain Kinase; PDE5 - Phosphodiesterase 5

Oral or vaginal use of sildenafil citrate has been proposed as a potential way to improve the endometrial receptivity and thickness.

Sher and colleagues in 2000 evaluated the effect of sildenafil, administered in the form of a vaginal suppository, 25 mg ,4 times per day on endometrial development in 4 women who had had thin endometrium during previous ART treatment (< 8 mm). Doppler studies revealed a decreased pulsatility index with sildenafil use, and in 3 of the 4 women a significantly thicker endometrium was achieved with the addition of sildenafil. All 3 of these women became pregnant.

Subsequently in a study by the same group using a larger cohort of women (n = 105) suffering from thin endometrium due to various causes as adenomyosis, endometritis or idiopathic, Seventy-three patients (70%) experienced an increase in their EMT to greater than 9 mm, and had a significantly higher implantation and ongoing pregnancy rates when compared to those who failed to respond (29% vs. 2%, P <0.01, and 45% vs. 0%, P < 0.01 respectively). The overall ongoing pregnancy rate in that cohort was 31.4% (33/105)(Sher G et.al. 2002)

In a comparative prospective study by **Check et.al. in 2004,** the effect of vaginal sildenafil citrate and vaginal estradiol valerate on EMT, blood flow, and pregnancy rates was compared in 16 patients who had failed to attain an EMT of 8 mm during previous FET cycle . They found no significant change in their EMT.

A RCT in 2013 **(Razieh et.al.**) did similar comparison between estrogen and sildenafil and found that endometrial thickness was significantly higher in the sildenafil citrate group (p<0.0001). They concluded that sildenafil citrate can be a good way to improve endometrial receptivity.

Many other studies concluded the beneficial effect of vaginal use of sildenafil in increasing the endometrial thickness **(Jyoti M et.al. 2016, Jerzak M et.al. 2008, Takasaki A et. al.2010)**

However, **Cochrane review ,2014** (Rosa B et.al.) on role of vasodilators for women undergoing fertility treatment concluded that evidence was insufficient to show that vasodilators increased the live birth rate in women undergoing fertility treatment. However, low-quality evidence suggests that vasodilators may increase clinical pregnancy rates in comparison with placebo or no treatment. The **vasodilators** considered in this review were nitric oxide donors (Nitroglycerin, Isosorbide Mononitrate), PTX, sildenafil. **Evidence was insufficient to show whether any particular vasodilator, administered alone or in combination with other active medications, was superior**

XIV. Role of L Arginine in thin endometrium

L-arginine, an amino acid is a precursor to nitric oxide, **(Moncada S 1993)** which increases blood flow by increasing the dilation of the arteries. There are not many studies on the role of L arginine for thin endometrium .

In a pilot study by **Takasaki et.al. (2010)** -Nine patients who showed a thin endometrium (<8 mm) in the late follicular phase were given l-arginine (6 g/day, orally), from the first day of the subsequent menstrual cycle until the day of hCG injection for ovulation induction. Endometrial thickness was measured at the day of hCG injection and was compared with that in the previous cycle without l-arginine treatment. L-arginine treatment improved EM in six (67%) out of nine patients, and one patient conceived.

Many more trials are needed before L arginine can be used for thin endometrium.

XV. Role of extended estrogen (how long to wait?)

In a study by **Chen et. al.** ,out of thirty-six women undergoing IVF with thin endometrium (<8 mm), 23 received fresh embryo transfer (control group), and 13 underwent frozen-thawed embryo transfer in the subsequent cycle after extended administration of exogenous estrogen (study group). In the study group, the mean endometrial thickness increased significantly from 6.7 mm in controlled ovarian hyperstimulation cycles to 8.6 mm after an extended estrogen therapy for 14 to 82 days with a mean of 30 days (P=0.031). Their pregnancy rate was significantly higher than that in the control group (38.5% vs. 4.3%, P=0.016).

In 2015, **Liu et al** conducted a study trying to adjust estrogen administration according to estradiol serum levels, starting with 18 mg/day of estrogen Valerate and keeping the estradiol levels between 600- 5000 pg/ml for long duration till the time 8 mm endometrium was achieved or adverse effects became obvious, 8 mm endometrial thickness was reached in 92.1% patients with long duration of estrogen treatment. The authors concluded that it is the duration of estrogen administration and not the serum concentration that matters.

Tourgeman et.al. (2001) demonstrated that the extended use of vaginal E2 (from 4 to 6 weeks) was successful in achieving adequate endometrial lining , but the presence of healthy endometrium is essential for the effect of estrogen.

Vaginal E2 could be particularly indicated for women who do not obtain sufficient endometrial thickness by other means. vaginal E2 administration improves endometrial thickening and uterine blood supply, acting on systemic and local level simultaneously (RJ Fanchin R 2001)

XVI. Role of stimulation in thin endometrium

Tamoxifen, a nonsteroidal antiestrogen agent, is widely used as adjunctive therapy for women with breast cancer. Tamoxifen is a selective estrogen receptor modulators (SERMs). Although the primary therapeutic effect of tamoxifen is derived from its antiestrogenic properties, this agent also has modest estrogenic activity. In standard dosages, tamoxifen may be associated with endometrial proliferation, hyperplasia. Some recent studies have shown that stimulation by Tamoxifen in patients having thin endometrium led to a significant increase in endometrial thickness. The endometrial effect of tamoxifen is associated with treatment duration, cumulative dose and possibly daily dose

Xin Chen et. Al. in 2013 published a case report of three cases of frozen embryo transfer with recurrent thin endometrium <6 mm and unresponsive to maximal extended estrogen therapy. They were managed successfully with tamoxifen treatment and all conceived. They suggested a dose of 20 mg daily of tamoxifen for five days.

Recent comparative study by **JanKe H et.al**.2017-showed that tamoxifen protocol improves EMT in patients after NC, HRT, and OI cycles during FET. Patients with PCOS show the most benefit from tamoxifen and achieve better pregnancy outcomes

In women undergoing OI with CC who have adequate follicular recruitment but thin endometrium (<7 mm), switching to tamoxifen in subsequent cycles improves endometrial thickness. **Kassey R.et.al. 2010**

A prospective study by **Wang et. Al. (2008)** on Ovulation induction with tamoxifen and alternate-day gonadotrophin in patients with thin endometrium showed significantly increased endometrial thickness ($P < 0.001$) and pregnancy rate ($P = 0.015$), decreased early miscarriage rate ($P = 0.001$) and thus improved ongoing pregnancy ($P < 0.001$) rate .They concluded that tamoxifen may not be a first-line treatment in patients with adequate endometrium,it may be a promising alternative for patients with thin endometrium.

XVII. Role of HCG in thin endometrium :

HCG receptors are present in the endometrium but the expression of functional receptors is regulated by the cyclical changes in the endometrium. **(Litcht P ,2001)** These receptors are also present in the proliferative phase. When HCG is administrated in the proliferative phase of endometrial growth during menstrual cycle there might be a positive interaction between endometrium and Human Chorionic Gonadotrophin.

Based on this hypothesis a proof of concept study was done by **Papanikolou Et Al in 2013.** In 17 Patients (recipient of fresh donor or frozen embryo) with thin endometrium subcutaneous injections of 150 IU HCG were initiated daily for 7 days on day-8 or 9 of the estrogen administration, and continuing

8 mg estrogen per day. After a week of HCG priming, (day-14 or 15) endometrial thickness was monitored with ultrasound, and progesterone was initiated. 35.3 % of the patients had more than 20 % improvement of their endometrial thickness after HCG priming. 17 % achieved an endometrial thickness more than 7 mm. interestingly even the two patients that had no response to the treatment achieved a pregnancy. This indicates a plausible positive paracrine effect of early HCG priming, days later during luteal phase, and related to the receptivity of the endometrium, regardless thickness.

Overall 41%(7/17) of them delivered concluding that HCG endometrial priming for 7 days in the proliferative phase with estrogen in frozen cycles seems highly promising, due to its positive effect on endometrial thickness and also on endometrial receptivity.

Similar study by **Robab Davar et.al.** in 2016 on 28 women with thin endometrium concluded that HCG priming of endometrium leads to significant improvement in thickness and pregnancy outcome.

XVIII. Can G-CSF instillation help in thin endometrium?

G – CSF is a type of colony stimulating factor which is produced by different tissues like endothelium, macrophages and a number of immune cells. It is found to play an important role in follicle development, ovulation and implantation. **(K.Yanagi S.et.al. 2002)**

Human endometrium contains a small number of mesenchymal stem like cells. These cells are thought to be responsible for cyclical growth of endometrium and reconstruction **(Schwab KE et.al., 2007)** Decreased endometrial stem cells in number and function may decrease endometrial growth. Adult stem cells have been identified in the highly regenerative human endometrium on the basis of their functional attributes. They can reconstruct endometrial tissue in vivo suggesting their possible use in treating disorders associated with inadequate endometrium. Intrauterine administration of bone marrow stem/ progenitor cells to a woman with thin endometrium refractory to estrogen stimulation was shown to regenerate her endometrium sufficiently to support a pregnancy **(Gargett CE et.al. 2011)**

It is proposed that G-CSF may stimulate Endometrial stem cells or mobilize bone marrow stem cells promoting growth of endometrium.

In 2011, E coli expression system was utilized for commercial production of recombinant human G -CSF called Filgrastim.

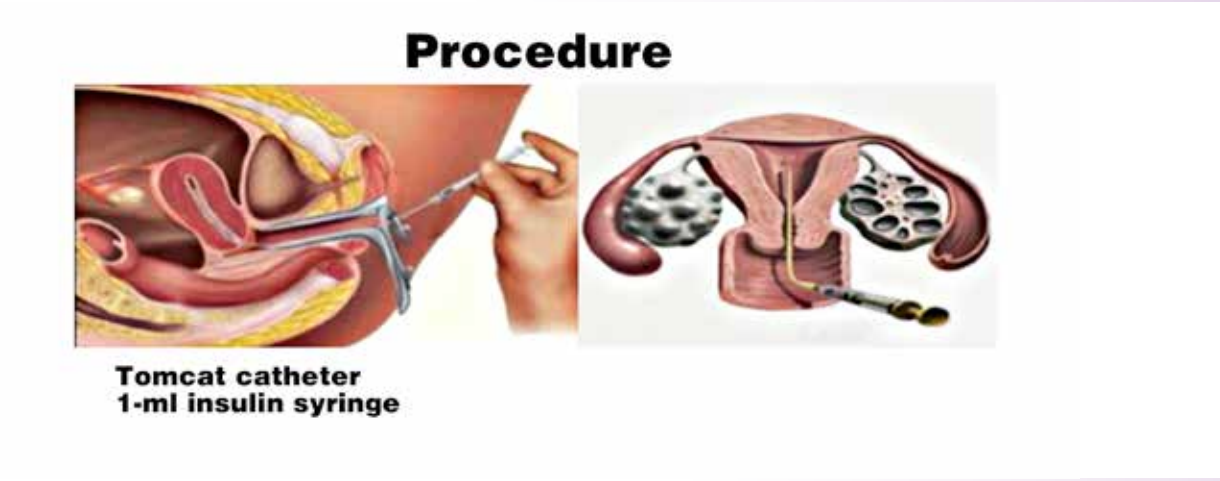
Gleicher et al in 2011 first demonstrated G-CSF as a novel remedy in patients with unresponsive, inadequate, and thin endometrium .He used intrauterine G-CSF in four patients undergoing IVF with thin endometrium after standard endometrial preparation. All the patients successfully underwent ET and conceived. They concluded that G-CSF treatment may, in general, improve IVF pregnancy chances. In their subsequent pilot cohort study, the investigators evaluated 21 subfertile women with thin endometrium. After instilling g-csf twice they reported that endometrial thickness showed improvement from 6.4 ±1.4 to 9.3 ±2.1 mm by the transfer day which was significant. Ongoing pregnancy rate was of 19.1% **(Gleicher N et.al. 2013)**

However in routine IVF patients G-CSf was not found to improve the pregnancy rates .In **2014 Barad Et al** did a study on routine unselected ivf patients and found no effect of G-CSF on endometrial thickness, implantation rates and clinical pregnancy rates . Another RCT by **Maryam Eftekhar, Et Al in 2016** showed that in normal IVF patients with normal endometrial thickness, the intrauterine infusion of G-CSF did not improve pregnancy outcomes.

Xie Y ET al (2017) in a recent meta-analysis included 11 studies and 683 patients concluded that G-CSF perfusion could significantly improve endometrial thickness, clinical pregnancy rate (risk ratio [RR]=2.52, 95% CI: 1.39-4.55), and embryo implantation rate (RR=2.35, 95% CI: 1.20-4.60), while it could decrease cycle cancellation rate (RR=0.38, 95% CI: 0.25-0.58) as compared to control group.

2RCTS by **M. Kunicki et.al. 2016** and **Fatemeh Sarvi 2017** also favoured the use of G CSF in patients with thin endometrium .

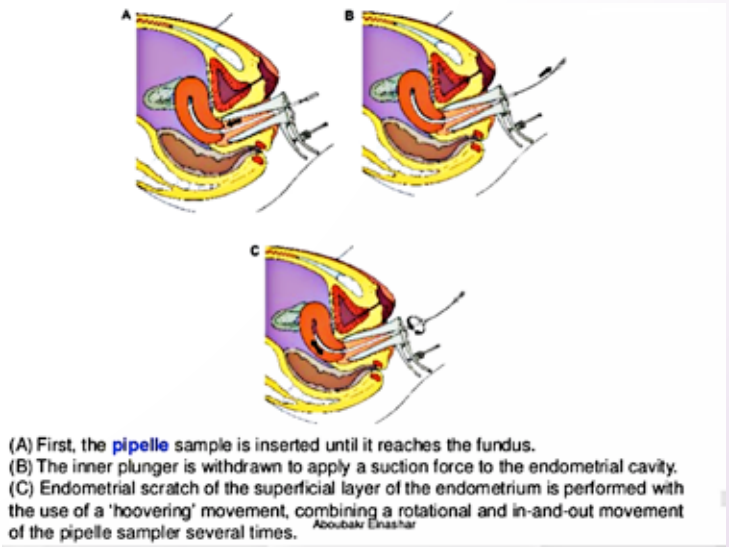
How to use G-CSF - Patients with a history of poor endometrium receive estrogen 6 mg per day and low dose aspirin 75 mg from day 2. On day 8-9 their endometrium is assessed. When the endometrial thickness is below 7 mm, 300 micrograms recombinant human G-CSF is instilled in the uterine cavity by IUI catheter. If endometrial thickness does not improve, instillation may be repeated. While instilling care should be taken not to injure the endometrial lining.



Although many trials have been in favor for using G - CSF in thin endometrium, it is still a new remedy. Effective dose, the day on which it is to be instilled and the efficacy of G-CSF in improving endometrium, and possibly pregnancy rates, needs to be confirmed in further randomized controlled trials. (Mohan Kamath et.al. 2015)

XIX. Role of endometrial scratching

Endometrial scratching, also known as endometrial injury, is a procedure undertaken to purposely disrupt the endometrium in women wanting to get pregnant. It is thought this disruption may somehow increase the chance of an embryo implanting, and therefore creating a pregnancy. **(Barash A et.al. 2003)** Endometrial injury induces an inflammatory reaction which favours implantation. Dendritic cells, natural killer cells and macrophages are employed to local injury and increased amounts of cytokines, chemokines and growth factors are secreted, thus resulting in successful implantation. **(Narvekar SA et.al. 2010)**



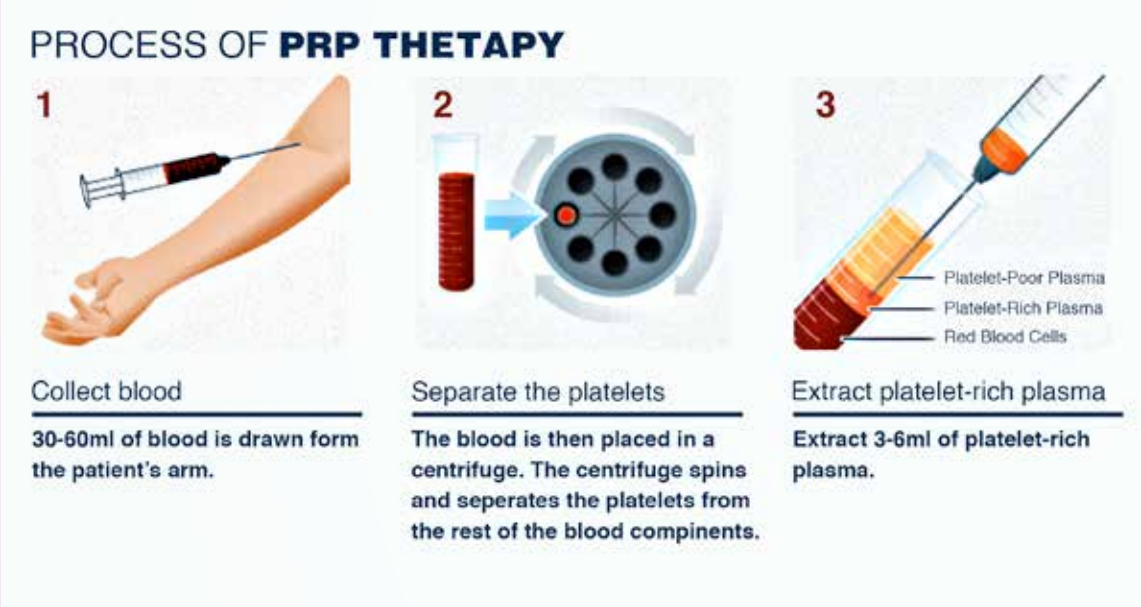
Many trials have demonstrated favourable effect of endometrial injury on implantation success rate, especially in women with recurrent implantation failure (RIF)**(Tiboni GM et.al. 2011).** A recent cochrane review which included nine randomised controlled trials with 1512 women suggest a benefit from endometrial scratching. **(Carolina et.al. Cochrane review, 2015)**

The hypothesis that scratching secretes some growth factors, which improves endometrial receptivity, should presumably work in cases of thin endometrium too in the regeneration of endometrium as it works in recurrent implantation failure cases. Instillation of G-CSF has shown promising results in growth of thin endometrium, which further supports the role of endometrial scratching in thin endometrium cases.

Xu Et al 2015 investigated the effect of scratching combined with G-CSF in thin ET patients and compared with patients with G-CSF alone. they established nominally higher, though no significant, Clinical pregnancy and live birth rate in combined group. Scratching did not impair G-CSF treatment but favoured pregnancy rate.

Further studies may help in coming to a conclusion regarding the role of endometrial scratching in patients with thin endometrium.

XX. Role of PRP (Platelets Rich Plasma) in thin endometrium



A new approach has been suggested for the treatment of thin endometrium in the form of Intra uterine infusion of platelet-rich plasma (PRP) **by Chang Y in 2015.** This pilot study was carried out on 5 women with refractory thin endometrium. They infused 0.5 ml of autologous PRP in uterine cavity on day 9/10 and again on day 13/14 of cycle while the patient was on estradiol valerate. There was satisfactory growth of endometrium in all 5 with 4 live births and 1 missed abortion.

Shahrzad Z et. al. in 2017 reported a similar pilot study on ten patients who had a history of refractory thin endometrium with PRP intrauterine instillation performed on day 11-12 and repeated on day 13-14. Endometrial thickness increased at 48 h after the first PRP and reached more than 7 mm after the second PRP in all patients. Embryo transfer was then carried out for all of them. Five patients were pregnant with 4 live births .

PRP is autologous blood plasma that has been enriched with platelets at about 4-5 times more than the circulating blood. It contains several growth factors such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factor (TGF) and other cytokines which help in tissue growth These growth factors release when platelets are

activated in the plasma. They regulate cell migration, attachment, proliferation and differentiation, and promote extracellular matrix accumulation. Growth factors and cytokines that may improve endometrial growth and receptivity. **(Chang Y et.al., Shahrzad Z et. al.)**

Platelet has been widely used for its tissue regeneration properties clinically in orthopedics, ophthalmology and wound healing etc. The effect of PRP in endometrial regeneration and in improving the inherent regenerating capacity of endometrium has been studied recently.

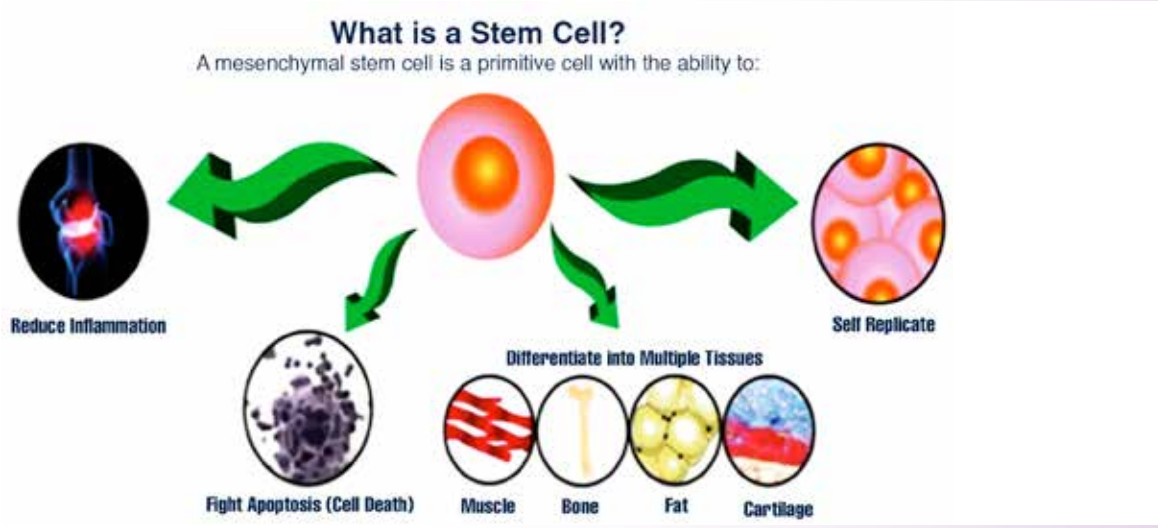
PRP is collected from autologous blood sample, so in comparison to G-CSF, PRP is more accessible and affordable with minimal risks of transmission of infectious disease and immunological reactions since it is made from autologous blood samples.

There is still very limited evidence regarding this new approach , so further larger well designed clinical trials are awaited before recommending generalized use of PRP for thin endometrium

XXI. Role of stem cell therapy-

Adult bone marrow is a known reservoir of stem and progenitor cells. The role of bone marrow-derived stem cells (BMDSCs) in the reconstitution of the human endometrium has been demonstrated by different groups of investigators. Many studies showed that the bone marrow derived stem cells are recruited in response to injury and are involved in the endometrial regeneration. The regenerative potential of stem cells was studied further in animal models with asherman syndrome and thin endometrium which gave promising results. **(Ikoma T Et.al. 2009 , Taylor B et.al. 2004)**

Recently **cervello and colleagues** (2015) demonstrated the regenerative capacity of stem cells by transplanting human CD133+ bone marrow-derived stem cells (BMDSCs) in an animal model of Asherman syndrome (AS). Human BMDSCs were mobilized by granulocyte-CSF injection (5 mg/kg/12 hours) subcutaneously during the course of 4 days. Five days later, isolation of CD133+ cells was performed by apheresis through peripheral venous access Engrafted cells were localized around endometrial blood vessels, inducing proliferation in surrounding cells.



In 2011 first human use of autologus endometrial angiogenic stem cells in a patient of asherman's syndrome was successful. **(CB Nagori et.al. ,2011)**

Recently in a human pilot trial, **Santamaria and colleagues (2016)** infused Bone marrow derived stem cells in the spiral artierioles in 11 patients with Asherman's syndrome resulting in increase in endometrial thickness and subsequent pregnancies.

Use of stem cells seem to be very promising in damaged and refractory endometrium. This therapy can become an alternative to surrogacy in many patients although the studies done are still very preliminary and more trials needed before stem cells can be applied in to clinical

practice

Conclusion -

Thin endometrium is a very frustrating problem faced by both doctors and patients. As such there is no defined cut off value below, which we label the endometrium as thin, but <7 mm has been commonly accepted. Also it has been observed that endometrial pattern rather than absolute thickness may be more important for implantation. Variety of therapeutic modalities (Extended estrogen therapy, low dose aspirin, sildenafil citrate, Arginine, vit E, pentoxiphylline, G-CSF intrauterine instillation, Tamoxifen etc.) have been tried to improve thin endometrium but none till today offers concrete answer to this perplexing problem. Newer modalities like platelet rich plasma instillation and stem cell therapy seem

Part -3

promising but need further larger well-designed trials before these can be advocated for routine use.

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Notes