

Prognostic significance of endometrial evaluation by ultrasonography in ovulation induced cycles

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ABSTRACT

Objective: To find out prognostic significance of pre-ovulatory endometrial evaluation by ultrasound scanning after exposure to the drugs during different protocols of ovulation induction with particular reference to clomiphene citrate and to correlate with pregnancy rate.

Methods: The thickness and texture of endometrium were the two biophysical parameters considered to be evaluated by ultrasonography. The cycles of 47 women of primary infertility were studied after ovulation induction by 4 types of protocols with or without clomiphene citrate.

Results: The pregnancy rate was recorded highest (36%, 5/14) in the exclusive treatment group of clomiphene citrate protocol. Most of the women in this group suffered with low level (<6 mm) endometrial thickness, but all five women who showed A-grade endometrium became

pregnant. The exclusive human menopausal gonadotrophin treated group and sequential protocol group achieved low pregnancy rates as 22% (2/9) and 27% (3/11). The luteinizing hormone releasing hormone analogue and follicle stimulating hormone protocol achieved 31% (4/13) pregnancy rate with more number of women showing A-grade endometrium quality.

Conclusion: The quality of endometrial texture rather than thickness of endometrium, can be considered as one of the strong prognostic factors to predict pregnancy rate in a majority of women.

Keywords: Ultrasonography, endometrium, ovulation.

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Implantation failure is one of the major problems posing the infertile population. The mechanisms of endometrial receptivity have not been clearly investigated due to lack of obligatory markers to identify the defects.¹ Moreover, very scanty information is available about the response and changes of endometrium to the drugs used in ovulation induction.

The advancement in the technology of high-resolution ultrasound (US) scanning by vaginal probe provides better visualization in non-invasive assessment of endometrium. Pre-ovulatory transvaginal sonography to determine endometrial status has been convincingly practiced at many

fertility centers. In fact, endometrium is the host substrate, which allows the embryo to oppose and invade into it. The biochemical attraction involved in the reciprocal excitement of these two intriguing elements is yet to be understood properly. Moreover, the endovaginal sonographic evaluation of endometrium is the only rapid-method of choice to predict implantation and most of the times, the prediction wins with successful pregnancy in IVF/ET cycles. The thickness and texture are the two important biophysical parameters of endometrium usually relied upon for the capacity to implant embryo. The endometrial thickness increases progressively and the normal endometrium measures

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more than 8 mm sonographically at the time of ovulation.² The multilayered (trilaminar/triple lined/hypoechoic) endometrium is considered as the most essential requirement to predict the outcome of pregnancy. Many workers believed that the sonographic multilayered pattern is a good indication of endometrial receptivity.³⁻⁵

In general, pregnancy outcome in assisted reproductive technology (ART) procedures is low, partly because of the detrimental effects of drugs used in ovarian hyperstimulation.⁶ Clomiphene citrate (CC) is a very old drug in use, as a promising agent of infertility patients for more than three decades. It is a non-steroidal antiestrogen compound which increases FSH and LH levels through its central action. The CC induction may result into abnormal endometrial histology.^{7,8} In addition, conflicting and controversial reports are existing about the actions of CC on endometrial thickness and texture.

The aim of the study was to find out the prognostic significance of pre-ovulatory endometrial evaluation on the day of HCG administration by ultrasound scanning after exposure to the drugs during different protocols of ovulation induction with particular reference to CC and to correlate with pregnancy rate.

Methods. Forty-seven women (age 32.3 ± 5.18 SD, range 23-36 years) of primary infertility, who had attended our fertility center were considered to admit into our study with the consent of the couples. The ovulation induction cycles (one cycle per each patient) were started by dividing them into four groups, as per the established induction protocols prepared for assisted reproductive technology (ART) program of our fertility center. All the patients in the 'CC-alone' group were advised to continue normal sexual life, with out applying any ART procedure, since all the husbands in this group were found normozoospermic without any known infertility problem. The pregnancy achieved in this group was termed as 'natural pregnancy'. But, the women in the other three groups were subjected to one of the ART procedures (IUI/IVF/GIFT/ICSI), since the semen profiles of the husbands in these groups were found low with different types of abnormalities and diagnosed as male factor infertility. The appropriate ART procedure to be applied was decided by the investigators, based on the medical history and infertility investigations of both wife and husband. The pregnancy achieved in these groups was termed as 'ART pregnancy'. The ovulation induction started after determining the base line hormonal profile with the evidence of serum E2 level less than 100 pmol/l. The E2 estimations and US scanning were made at regular intervals to monitor the cycles.

Ovulation induction. The first group (14 cycles) were induced exclusively with CC (Clomid, Marri-

Merrell Dow, Italy), 100 mg/day in two divided doses starting from day-3 cycle after reaching the base line hormonal profile. The dose was continued for 5-7 days so that the leading follicle would achieve the required size at the proper time. The second group (9 cycles) were induced with HMG alone (Pergonal, Serono Laboratories, Randolph, USA). The dose of HMG was adjusted individually 1-4 ampoules/day in order to achieve leading follicle growth to the required size. The third group (11 cycles) were administered the drugs sequentially, first with CC (100 mg/day) in two divided doses as that of first group and the day following the fifth day of CC administration, HMG was continued instead of CC. The dose of HMG (1-4 ampoules/day) was adjusted in order to achieve leading follicle growth to required size. The fourth group (13 cycles) were down regulated by long protocol using LH-RH analogue (Goserelin, Zoladex, of Zeneca) cylindrical depot 3.6 mg/s.c from day-21 of the previous cycle. Then, they were stimulated with FSH (Metrodin, Serono Laboratories, Rome, Italy) 2-3 ampoules/day for 7-10 days in the subsequent cycle.

Transvaginal ultrasonography. Apogee,[®] 800 plus machine (ATL) with 5-9 MHz dynamic frequency curvilinear vaginal transducer was used for all the measurements. A complete gynecological US scanning was performed initially, so as to rule out any pathological abnormality. The scanning has been repeated at regular intervals to monitor the ovulation induction cycles. Again, the ultrasonic observations were made on the day before HCG administration. The consideration of sonographic texture of endometrium before HCG injection during ART procedures, is ideal to correlate the pregnancy rate.⁹⁻¹¹ In addition to the follicular assessment, the uterus was also scanned in the longitudinal and transverse planes. The endometrium was imaged in long axis and measured at its maximum thickness. This measurement included both layers of anterior and posterior echogenic interfaces between endometrium and myometrium. The readings of endometrial thickness and texture were recorded simultaneously in all the cases. Finally, the obstetrical scanning was done at 6 weeks of ART procedure to confirm the intrauterine viable pregnancy.

Results. Thickness and texture of endometrium were the two different creative entities depicted clearly by the high resolution US scanning through vaginal probe. The texture (presence and absence of multilayered endometrium) was classified into three distinct grades (Figure 1, 2 and 3). A-grade was represented by the central hypoechoic core in between the prominent echogenic lines usually seen in the late proliferative stage (before ovulation). The size and distribution of endometrial glands are said to be uniform in the A-grade pattern. B-grade was

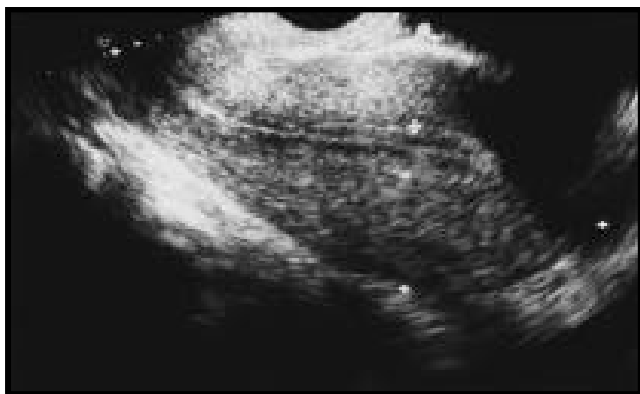


Figure 1 - (A-Grade) Central hypochoic core with prominent echogenic lines in between, representing multilayered endometrial texture.



Figure 2 - (B-Grade) Central hypochoic core without echogenic lines in between, representing the stage of ill development of multilayered endometrial texture.



Figure 3 - (C-Grade) Homogeneous hyperechoic picture, representing no development in the central hypochoic core of endometrial texture.

showed by central hypoechoic texture with out echogenic lines. C-grade was depicted by homogeneous hyperechoic picture. The size and distribution of glands are expected to be variable and heterogeneous in C-grade, whereas in-between stage in B-grade patterns. The reflection of ultrasonic waves in the sonographic study depends on the pattern of distribution of endometrial glands and the stromal cell population. The sonographic texture is positively correlated to histological and morphological findings.¹² Similarly, the endometrial thickness was also classified into three sub-classes as <6 mm, 6 to 13 mm, and >13 mm. The limits of thickness were fixed on either side as <6 mm^{13,14} and >13 mm⁵ based on the good pregnancy rate experienced between these limits in the elsewhere studies. In our study, the pregnancy outcome was correlated with texture (Table 1) and thickness (Table 2) of endometrium in different ovulation induction protocols.

Ovulation induction with clomiphene citrate alone. The natural pregnancy rate was recorded highest (36%, 5/14) in this group. However, more cases (7/14) of C-grade texture and low (<6 mm) thickness (9/14) of endometrium were recorded out of 14 cycles. All the pregnancies were achieved only in A-grade with the thickness of 6 to 13 mm endometrium. No pregnancy was observed in other sub-classes of texture and thickness.

Ovulation induction with human menopausal gonadotrophin alone. The lowest pregnancy rate (22%, 2/9) was recorded in this group. Similar to the 'CC alone' group, the pregnancies were achieved only in the sub-class of A-grade endometrium with 6 to 13 mm thickness. However, A-grade and B-grade endometrial textures on the day of HCG administration were seen in four patients each, whereas C-grade was seen in only one patient in this group. Two patients showed low levels of thickness (< 6 mm) and one patient showed high level of thickness (> 13 mm) but no one became pregnant in these two sub-classes.

Ovulation induction of sequential protocol with Clomiphene Citrate and human menopausal gonadotrophin. Two pregnancies with A-grade and one pregnancy with B-grade endometrial texture were recorded in this group. All the three pregnancies were found with the 6 to 13 mm preovulatory endometrial thickness. No pregnancy was achieved in the other two sub-classes of endometrial thickness. The pregnancy rate was calculated as 27% (3 out of 11 cycles) in this group.

Ovulation induction with luteinizing hormone releasing hormone analogue and follicle stimulating hormone. The pregnancy rate (31%, 4/13) recorded in this group was placed next to 'CC alone' group (36%, 5/14). Three pregnancies in A-grade and one pregnancy in B-grade were observed. The pregnancies were distributed in all the three sub-

Table 1 - Endometrial texture and ovulation induction protocol in relation to pregnancy outcome.

Ovulation induction protocol (Total patients number)	Endometrial texture			Pregnancy rate
	A Grade (Preg no)	B Grade (Preg no)	C Grade (Preg no)	
CC alone (14) (natural pregnancy)	5 (5)*	2 (0)*	7 (0)*	35.71
HMG alone (9) (ART pregnancy)	4 (2)*	4 (0)*	1 (0)*	22.22
Sequential protocol (11) with CC and HMG (ART pregnancy)	2 (2)*	5 (1)*	4 (0)*	27.27
LH-RHa and FSH (13) (ART pregnancy)	9 (3)*	2 (1)*	2 (0)*	30.76
*Number of pregnancies achieved in that particular group Natural pregnancy: Pregnancies achieved by natural way of sexual life with out any ART procedure ART pregnancy: Pregnancies achieved through assisted reproductive technology (ART) procedures HMG:human menopausal gonadotrophin LH-RHa: luteinizing hormone releasing hormone analogue FSH: Follicle stimulating hormone				

classes of endometrial thickness. Surprisingly, one pregnancy was achieved with low thickness (< 6 mm), and two in medium thickness (6 to 13 mm) and one in high thickness (> 13 mm) of endometrium. This is the only induction protocol which recorded pregnancies in the low (<6 mm), as well as high (>13 mm) endometrial thickness groups. But, pregnancy could not be achieved with C-grade endometrial texture, just like the other three groups.

In general, no pregnancy was achieved with C-grade endometrial texture by any type of induction

protocol used in this study. On the other hand, pregnancies were also not achieved with B-grade and C-grade texture by single drug (CC or HMG) induction protocols. However, more number of pregnancies (12 out of 20) were achieved with A-grade endometrial texture. Similarly, more number of pregnancies (12 out of 25) were achieved with endometrial thickness ranging 6 to 13 mm group.

Statistical evaluation was not performed because of the small sample size and in such cases the derived

Table 2 - Endometrial thickness and ovulation induction protocol in relation to pregnancy outcome.

Ovulation induction protocol (Total patients number)	Endometrial thickness in mm			Pregnancy rate
	<6 (Preg no)	6 to 13 (Preg no)	>13 (Preg no)	
CC alone (14) (Natural pregnancy)	9 (0)*	5 (5)*	0 (0)*	35.71
HMG alone (9) (ART pregnancy)	2 (0)*	6 (2)*	1 (0)*	22.22
Sequential protocol (11) with CC and HMG (ART pregnancy)	3 (0)*	8 (3)*	0 (0)*	27.27
LH-RHa and FSH (13) (ART pregnancy)	4 (1)*	6 (2)*	3 (1)*	30.76
*Number of pregnancies achieved in that particular group Natural pregnancy: Pregnancies achieved by natural way of sexual life with out any ART procedure ART pregnancy: Pregnancies achieved through assisted reproductive technology (ART) procedures HMG:human menopausal gonadotrophin LH-RHa: luteinizing hormone releasing hormone analogue FSH: Follicle stimulating hormone				

statistical significance may not be meaningful. However, it is quite clear in our data to ascertain significant outcome of the results.

Discussion. Implantation depends on the interactions of a large number of variables and molecular factors. The failure of pregnancy is predominantly due to poor quality oocyte and bad endometrial receptivity.¹⁵ The proliferative endometrium will be prepared well when it is primed optimally with steroids. Proliferative and secretory changes of endometrium are very much required for the proper implantation and placentation. The antiestrogenic effect of CC has been demonstrated^{16,17} sonographically as thinner endometrium. Women, who received CC for more than three consecutive cycles showed the sonographic evidence of reduced endometrial thickness¹⁰ probably due to its persistent effect on endometrial estrogen receptors. The same effect may be seen even with higher doses (> 100 mg/day) in a single cycle. In the present study the CC was used only for one cycle of each patient and the dose did not exceed 100 mg per day. Five out of fourteen patients were demonstrated with 6 to 13 mm thickness and the remaining nine showed <6 mm thickness of endometrium with 'CC alone' protocol. Interestingly, all the five patients of 6 to 13 mm thickness became pregnant. Eight out of eleven patients achieved in the same range of thickness (6 to 13 mm) with sequential protocol of CC and HMG, but only three out of eleven in the same sub-class became pregnant. Recently, intrauterine pregnancy is established even with the thickness of endometrium less than 4 mm.¹⁸ Therefore, the prognostic value of endometrial thickness is no more a strong predictor of pregnancy.

The multilayered sonographic endometrial texture is a reliable marker to predict implantation and pregnancy in a majority of population undergoing ART procedures.^{2,4,5,10} We have also experienced the same result that twelve out of twenty pregnancies were achieved in the A-grade multilayered endometrial texture in our study. We agree with the statement that the good endometrial texture is a reliable marker to predict pregnancy, provided the other conditions are fulfilled. The influence of CC can be reduced by either lowering the dose or continuing HMG in a sequential protocol.¹⁹ Sequential CC and HMG therapy has become an increasingly utilized method of ovarian stimulation for patients who failed in the CC alone treatment.^{20,21} Conversely, in our study lower pregnancy rate (27%, 3/11) in the sequential protocol with CC and HMG was obtained than that of 'CC alone' protocol (35.71 %, 5/14). Still lower rate of pregnancy (22%, 2/9) was achieved with 'HMG alone' protocol. The use of HMG also proved elsewhere, with a high rate of abnormal endometrial development and detrimental effects on endometrial receptivity.²² The inhibitory

effect of HMG on the functional activity of endometrium, if not on its morphological development, is not well explicit. No pregnancy was achieved in the C-grade endometrial texture by means of any ovulation induction protocols implemented in this study. However, two pregnancies, one each in sequential protocol and LH-RHa protocol were obtained with B-grade endometrial texture. The gonadotrophin releasing hormone analogues have been proved to be free of any detrimental effects on peri-implantation endometrium.²³ This can also be attributed to the fact that superior quality embryos might implant even if the endometrial pattern is of some what poor quality. Moreover, pregnancy outcome depends on multifactorial complexity rather than a single milieu of endometrial profile.

We conclude that the endometrial texture on the day of HCG administration can be safely considered as one of the strong prognostic predictors in a majority of women undergoing ART procedures as well as in the natural cycles, since the multilayered US picture implies the maturational development of endometrium. The endometrial thickness is no more a strong predictor of pregnancy. The potentiality of endometrium for the implantation and maintenance of pregnancy certainly depends on the rich quality of texture detected by sonographic evaluation.

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