

Randomized Double Blind Placebo-controlled Study to Evaluate the Role of M2-Tone as an Adjuvant Treatment in IUI Protocol by Assessing its Effects on Endometrial Morphology, Vasculature and Pregnancy Rate Using 3D Color Doppler in Female Infertility

¹Narendra Malhotra, ²Jaideep Malhotra, ³JP Rao, ⁴Shobhna Arora, ⁵Neharika Malhotra, ⁶Randhir Puri

¹Infertility Specialist, Department of Obstetrics and Gynecology, Malhotra Nursing and Maternity Home (P) Ltd, 84, Mahatma Gandhi Road, Agra, Uttar Pradesh, India

²Infertility Specialist and ART Specialist, Department of Obstetrics and Gynecology, Malhotra Nursing and Maternity Home (P) Ltd, 84, Mahatma Gandhi Road, Agra, Uttar Pradesh, India

³Ultrasonologist, Malhotra Nursing and Maternity Home (P) Ltd, 84, Mahatma Gandhi Road, Agra, Uttar Pradesh, India

⁴Counsellor, Malhotra Nursing and Maternity Home (P) Ltd, 84, Mahatma Gandhi Road, Agra, Uttar Pradesh, India

⁵Senior Resident, Malhotra Nursing and Maternity Home (P) Ltd, 84, Mahatma Gandhi Road, Agra, Uttar Pradesh, India

⁶Consultant, Malhotra Nursing and Maternity Home (P) Ltd, 84, Mahatma Gandhi Road, Agra, Uttar Pradesh, India

Correspondence: Narendra Malhotra, Infertility Specialist, Department of Obstetrics and Gynecology, Malhotra Nursing and Maternity Home (P) Ltd, 84, Mahatma Gandhi Road, Agra, Uttar Pradesh-282010, India, e-mail: mnmhagra3@gmail.com

Abstract

Objective: To compare the efficacy of M2-Tone, a phytopharmaceutical, and placebo as an adjuvant in IUI protocol in improving endometrial receptivity as assessed by endometrial morphology, vascularity and pregnancy outcome in female infertility using the 3D color Doppler technique.

Design: Double blind placebo controlled comparative trial.

Setting: Outpatient clinic of MTTBC (Malhotra Test Tube Baby Center), Agra in India.

Patient(s): Fifty-five patients with infertility were recruited. Twenty-eight patients were administered tablet M2-Tone and twenty-seven patients were given placebo. Both drugs were given orally in the dose of 2 tablets twice a day for three months prior to the IUI procedure.

Intervention(s): M2-Tone, placebo, clomiphene citrate, hCG, 3D Color Doppler, IUI.

Main outcome measure(s): Endometrial thickness, endometrial lining, endometrial vascularity, sex steroids and pregnancy rates.

Result(s): Out of total 55 patients only 50 patients completed the study. In M2-Tone group endometrial thickness increased to mean 8.65 mm from 4.6 mm at baseline (Day 2 of menstrual cycle) whereas in placebo group it rose to mean 6.89 mm from 4.8 mm at baseline. The decrease in the mean score of uterine artery blood flow impedance (Pulsatility index PI) was 1.13 in M2-Tone group and 0.72 in placebo group. The uterine score in the M2-Tone group increased significantly from 3.15 to 13.31 and from 3.16 to 10.08 in placebo group. There was corresponding increase in pregnancy rate in the M2-Tone group (30.77%) as compared to the placebo group (16.66%). Though there was slight increase in the serum estradiol and progesterone levels in M2-Tone group compared to the placebo group the difference was not statistically significant.

Conclusion(s): M2-Tone has shown better endometrial thickness, endometrial layering and better color flow to zone 3 of the endometrium compared to placebo. M2-Tone as an adjuvant treatment in IUI protocol showed significant improvement in pregnancy rate. This beneficial effect of M2-Tone can be attributed to improvement in the endometrial vascularity and morphology in the subjects by its modulating action on the hormonal status.

Keywords: M2-Tone, clomiphene citrate, endometrial vascularity.

INTRODUCTION

Regardless of advances in assisted conception, female infertility remains one of the major challenges for intrauterine insemination (IUI) and *in vitro* fertilization (IVF). The incidence of female

infertility is about 10 to 15%. The majority of these couples opt for IUI and IVF. But the overall success rate of IUI in terms of clinical pregnancy per cycle is about 10 to 20% and about 25 to 40% in patients going for IVF.¹⁻⁴ The success of implantation

process depends upon several conditions. The embryo must be healthy and should have reached the blastocyst stage. A receptive endometrium and a molecular dialogue between it and the embryo are also prerequisites. Endometrial receptivity is a self-limiting period in which the endometrial epithelium acquires a functional and transient ovarian steroid-dependant status that allows blastocyst adhesion. The endometrium is controlled by the ovarian sex hormones estrogen and progesterone, which elicit their actions by binding to specific high affinity receptors.⁵

Ayurveda, the ancient Indian medical science, has identified four requisites for normal healthy pregnancy. They are – the season (normal ovarian steroid-dependant status), the good quality viable seeds (healthy gametes, i.e. sperm and ovum), the fertile soil (healthy and conducive endometrial structure) and the water (normal endometrial vasculature). Poor quality of any of these four essentials leads to infertility.

M2-Tone, an herbal medicine by M/s Charak Pharma Private Ltd, Mumbai has established its estrogenic effect in female infertility through various studies conducted in the past. A preliminary study by Joglekar SN et al indicated estrogenic effect of M2-Tone evidenced by increase in weight and glycogen content of uterus.⁶ Pattanayak M et al compared the efficacy of Syrup M2-Tone with low dose aspirin and an antioxidant as an adjuvant in IVF cycles for poor responders. The results of the study demonstrated the advantage of Syrup M2-Tone over the others as far as implantation rates and live birth rates are concerned. Parulekar S et al, Dubey P et al, Gupta I et al, Kotadawala P et al and Urala MS et al in five separate clinical studies showed improvement in menstrual irregularities.⁷⁻¹¹ Tanksale VS et al in a clinical trial showed that Syrup M2-Tone stimulates ovulation in anovulatory females.¹² Chandravati et al after a clinical evaluation reported that Syrup M2-Tone achieved a very good rate of conception (33.3%) in infertility.¹³ In an earlier clinical trial by Malhotra N et al Syrup M2-Tone showed significant improvement in endometrial morphology and increase in pregnancy rate.¹⁴ An animal study conducted by Bhutani K et al to evaluate the efficacy and safety of Lodhra one of the main ingredients of M2-Tone revealed enhanced folliculogenesis, presence of mature follicles and detached oocytes as a result of increased FSH and LH levels.¹⁵

Thus Syrup M2-Tone has shown its beneficial effects on the three prerequisites (menstruation, ovum, and endometrium) for conception and only the effects on endometrial vasculature remain to be evaluated. This originated the idea of conducting

a clinical trial to evaluate the effect of tablet. M2-Tone on improving the endometrial vasculature.

We hypothesize that adding M2-Tone prior to starting the IUI protocol can promote normal endometrial proliferation, perfusion and improve endometrial receptivity for better implantation in the subjects. In the present study, we used the Color Doppler and 3D power angiography technique for noninvasive evaluation of our hypothesis. This study, therefore, aimed to evaluate the role of orally administered M2-Tone in modifying uterine vascular parameters, endometrial thickness, sex steroids and the corresponding pregnancy rate in Indian population.

MATERIALS AND METHODS

This was a double blind placebo controlled randomized study. Total 55 infertile women who approached MTTBC (Malhotra Test Tube Baby Centre), Agra and underwent IUI programs were studied for 3 months.

Informed consent was taken from each subject prior to the study. The demographic parameters of the subjects are mentioned in Table 1. The subjects who were included in the study had regular menstrual cycles (28 ± 4 days).

Those with a history of pelvic or gynecological surgery, especially related to uterus or endometrial cavity were not included in the study. Subjects with a history of previous pelvic inflammatory disease, endometrial tuberculosis, etc. which have influence on the basic structural or functional endometrial pattern and with heavy menstrual bleeding were excluded from the study. Other exclusion criteria were body mass index [BMI > 30], endometriosis, and deep dyspareunia.

After a 3 months 'wash-out' period before initiating the study all the patients were evaluated in one natural cycle for their hormonal levels. After overnight fasting the peripheral blood was obtained and analyzed for plasma estradiol and plasma progesterone levels using radioimmunoassay. These readings were considered as baseline hormonal parameters. After proper counseling patients were administered either M2-Tone tablets or identical placebo for next 3 months prior to IUI protocol. The patient's treatment was assigned centrally by block randomization; one group received 2 tablets of M2-Tone twice daily for 3 months and the other group received an identical placebo therapy on similar lines before the initiation of IUI protocol.

As per the IUI protocol, ovarian stimulation was achieved in both the groups by administration of antiestrogenic drug (Clomiphene citrate) daily for 5 days starting from the second

Table 1: Demographics and cycle characteristics for the two groups

Parameter	M2-Tone	Placebo	P value
Age (years)	28.5 ± 6.5	27.91 ± 9.75	NS
Body mass index (kg/m ²)	25.95 ± 0.95	25.01 ± 0.05	NS
Mean duration of infertility (years)	6.6 ± 3.8	6.2 ± 4.7	NS
Follicular phase (days)	14.28 ± 0.39	14.08 ± 0.75	NS
Luteal phase (days)	14.13 ± 0.59	14.52 ± 0.63	NS
Total cycle length (days)	27.93 ± 0.32	27.03 ± 1.85	NS

NS: Not significant

day of menstrual cycle. When at least one follicle measuring between 16 to 18 mm in diameter was present 5,000 IU human chorionic gonadotrophin (hCG) injection was administered intramuscularly and IUI was performed within 24 hours after evidence of ovulation. During the entire study regimen the patients were submitted (day 0, day 2 of MC, day of follicle measuring 14 mm, day of hCG administration, day of IUI) to Doppler evaluation for assessing endometrial thickness, endometrial lining, myometrial echogenicity, uterine artery flow index, and endometrial blood flow index. The plasma estradiol levels were assessed on the day of maximum endometrial thickness and progesterone levels were assessed on the 7th day after ovulation. All the patients were evaluated for three cycles.

DOPPLER EXAMINATIONS

Doppler flow measurements of uterine, endometrial and myometrial artery blood flow were performed transvaginally with 6.5 MHz color Doppler technique (Fig. 1). All the patients were asked to relax for at least 15 minutes before being scanned, and were asked to completely evacuate the bladder to minimize any external effects on blood flow. A 50 Hz filter was used to eliminate low frequency signals originating from the vessel wall movements. The probe was directed into the vaginal fornix to identify the ascending branch of uterine artery. A color flow transvaginal images of the ascending branches of the uterine artery was taken bilaterally in a longitudinal plane. The endometrial morphology measurements were performed on the longitudinal section of the uterus. The angle of insonation was altered to obtain the maximum color intensity. Placing the sample volume across the vessel and entering the pulsed Doppler mode produced good color signals and blood flow velocity waveforms thus obtained were recorded. The pulsatility index (PI = S-D/mean), defined as the difference between the peak systolic (S) and end diastolic (D) flow velocity divided by the mean flow velocity was calculated electronically. The PI has been shown

to reflect blood flow impedance and may be used when the end diastolic frequency shift is absent or reversed. For each examination the mean value of three consecutive waveforms was obtained. No significant difference between the PI of the left and right uterine arteries was observed. Therefore, the average value of both arteries was used. The Doppler sonologist who was unaware of the patient treatment group performed Doppler analysis.

UTERINE SCORING SYSTEM

In this study to assess the uterine receptivity we analyzed all the ultrasonographic and Doppler parameters. We used the slightly modified version of Applebaum Uterine Scoring System. This uterine scoring system (Table 2) includes all the following parameters: endometrial thickness, endometrial lining (pattern), myometrial echogenicity, uterine artery pulsatility index (PI), and endometrial artery blood flow (vasculature). Each parameter is scored as shown in Table 2.

Table 2: The uterine scoring system¹⁶

S. No.	Parameter	Score
1.	Endometrial thickness	
	< 7 mm	0
	≥ 7 mm	3
2.	Endometrial lining	
	Multilayered endometrium	3
	Others	0
3.	Myometrial echogenicity	
	Homogeneous	1
	Nonhomogeneous	0
4.	Uterine artery pulsatility index (PI)	
	≤ 3	4
	> 3	0
5.	Endometrial blood flow	
	Present	3
	Absent	0
Total		14

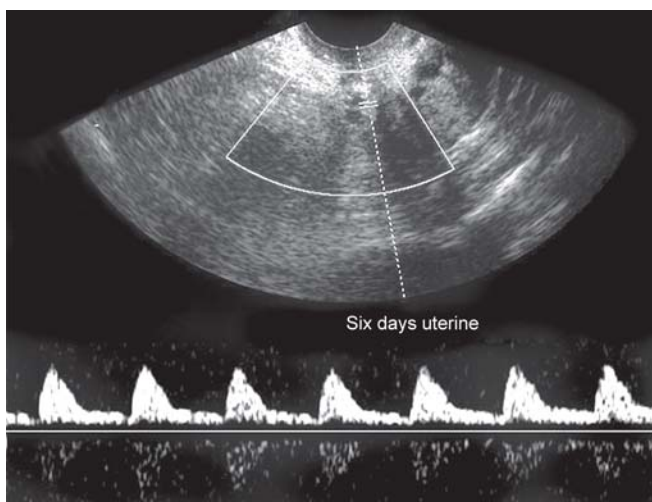


Fig. 1: Normal uterine artery

Endometrial Morphology in View of Uterine Scoring System

The maximum thickness of the endometrium was measured as the maximum distance between each myometrial/endometrial interface through the central longitudinal axis of the uterus.

Endometrial vascularization was assessed on a longitudinal scan of the uterus. The vascularization was considered as positive and scored 3 if more than three vessels penetrating the outer hypoechoic area surrounding the endometrium could be seen.

Endometrial Vascularity/Perfusion

The pulsatility index (PI = S/D) defined as the difference between peak systolic (S) and end diastolic (D) flow velocity divided by the mean flow velocity was calculated electronically by the machine. The PI has been shown to reflect blood flow impedance.

HORMONAL ASSAY

Peripheral blood was obtained after overnight fast on the same day that Doppler examination took place. Blood was immediately centrifuged and serum stored at -20°C, and estradiol and progesterone values were measured. Clinical pregnancies were detected by serum b-hCG levels in at least two determinations 12 to 14 days after IUI and were confirmed by ultrasonographic screening of the gestational sac showing embryo activity 14 days after the last b-hCG determination.

Results

Out of total 55 patients 50 completed the study. After the blinding code was disclosed, it was found that two patients from M2-Tone group and 3 patients from the placebo group were lost to follow-up and none of the remaining reported any adverse effects. The records of 26 patients in the M2-Tone group and 24 patients in the placebo group were considered for final analysis.

ENDOMETRIAL MORPHOLOGY AND UTERINE HEALTH

As compared to placebo M2-Tone has shown better proliferative effect on endometrial thickness and layering and better color flow to zone 3 of the endometrium (Fig. 2).

The endometrium developed into three line endometrium pattern with the progress from follicular phase to just before ovulation. Table 3 indicates the results of all the uterine parameters evaluated using 3D color doppler.

The mean endometrial thickness increased significantly from 4.6 mm at baseline to 8.65 mm in M2-Tone group whereas in the placebo group there was increase from 4.8 mm to 6.89 mm (Fig. 3).

Uterine artery flow index, which was calculated using mean pulsatility index (PI) reduced in M2-Tone group to 1.99, whereas it decreased slightly in the placebo group to 2.37 as compared to their respective baseline readings (Fig. 4). This indicates increase in the blood flow to the endometrium in M2-Tone group. The mean uterine score in M2-Tone group was 13.31 as compared to 10.08 in placebo group.

The decreased blood flow impedance (PI) and increased systolic and diastolic arterial blood flow indicates good endometrial perfusion in M2-Tone group as compared to the placebo group (Figs 5 and 6).

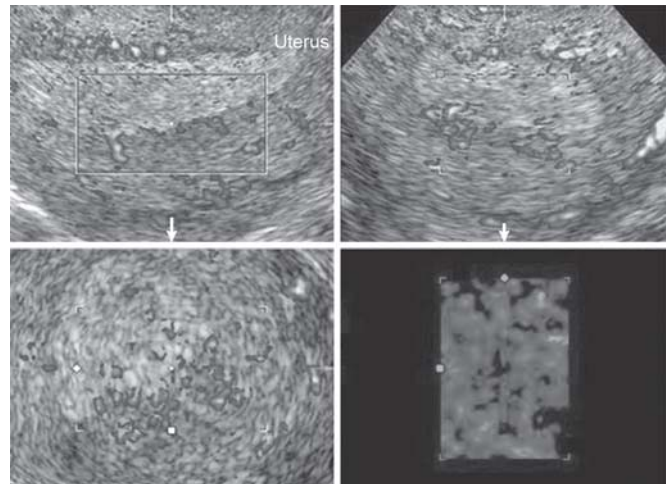


Fig. 2: Increased blood flow to the endometrium in the M2-Tone group

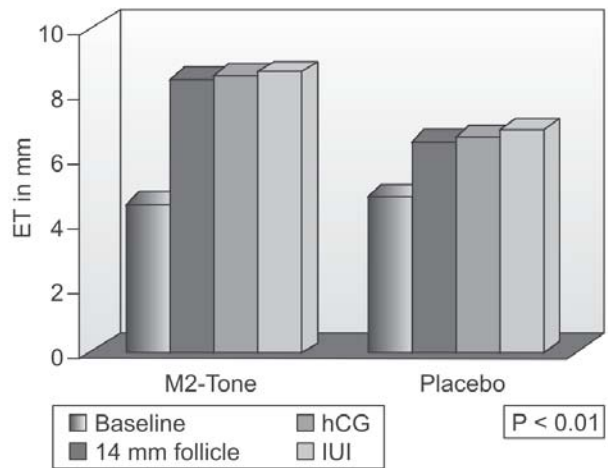


Fig. 3: Comparative evaluation of increase in endometrial thickness (ET) in M2-Tone and placebo group

HORMONAL ASSAY

In the M2-Tone group the serum estradiol levels increased from mean 800.84 pmol/l to 1162.69 pmol/l and in the placebo group they increased from 788.02 pmol/l to 1144.53 pmol/l. The progesterone levels increased from mean 56.26 nmol/l to 81.59 nmol/l in M2-Tone group and in placebo group they

Table 3: Comparison of ultrasound parameter for endometrial receptivity between two study groups

S. No.	Parameters	Score in M2-Tone group				Score in placebo group			
		Baseline mean	14 mm follicle	hCG	IUI	Baseline mean	14 mm follicle	hCG	IUI
1.	Uterine score	3.15	8.15	13.31	13.31	3.16	8.67	10.67	10.08
2.	Endometrial thickness (mm)	4.60	8.48	8.58	8.65	4.8	6.54	6.64	6.89
3.	(PI)	3.12	2.20	2.10	1.99	3.09	2.87	2.72	2.37

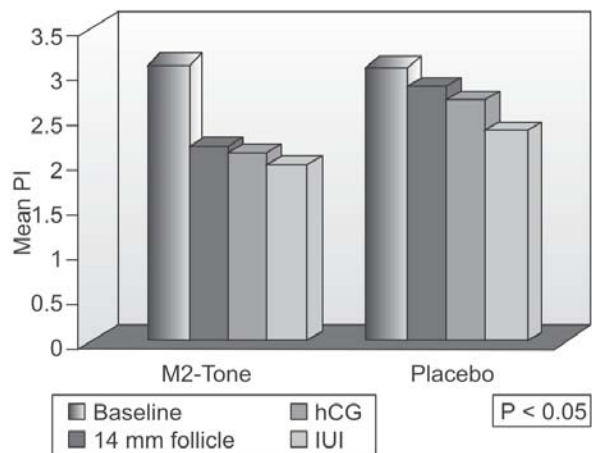


Fig. 4: Comparative evaluation of decrease in pulsatility index (PI) in M2-Tone and placebo group

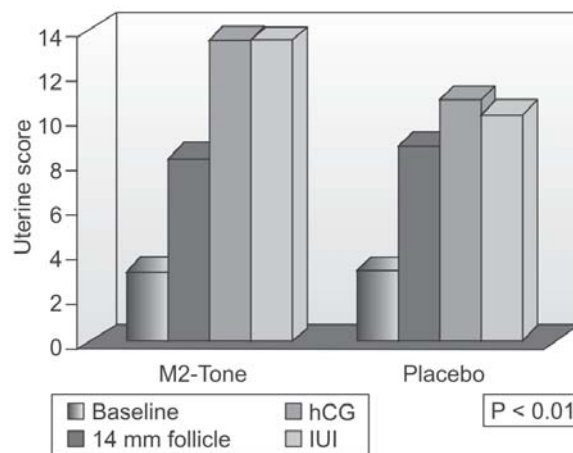


Fig. 7: Comparative evaluation of increase in uterine score in M2-Tone and placebo group

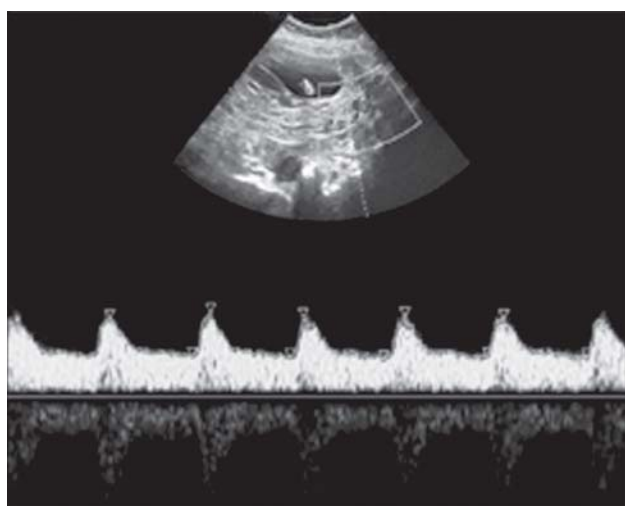


Fig. 5: Wave form in M2-Tone group shows disappearance of notch and good diastolic flow

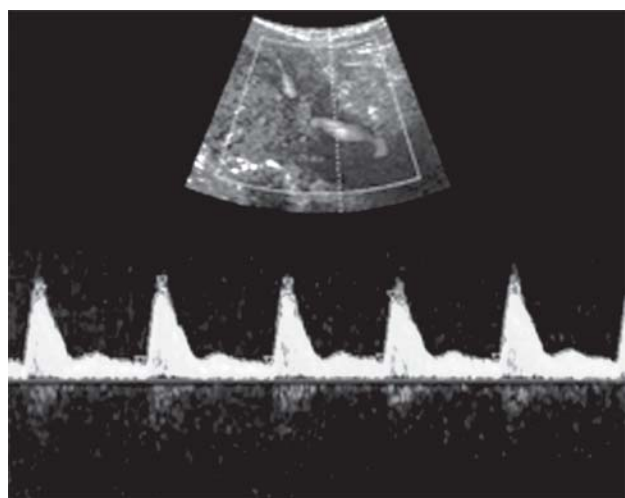


Fig. 6: Wave form in placebo in group shows a notch in early diastole and a small amount of diastolic flow

increased from 54.87 nmol/l to 69.09 nmol/l. The serum estradiol and serum progesterone levels were slightly higher in M2-Tone group but the difference between the two groups was not statistically significant.

Based on the uterine scoring system, the pregnancy outcome was calculated. No pregnancy occurred if the uterine score was between 0 and 10 ranges. The pregnancy rate in M2-Tone group was 30.77 % and 16.66 % in placebo group. With a score of 11-15 there was a 31% chance of pregnancy in M2-Tone group (Fig. 7). The clinically evaluated pregnancy outcome coordinated well with the pregnancy outcome calculated on the basis of uterine scoring system.

PREGNANCY OUTCOME

There was a significant increase in the uterine score in M2-Tone group as compared to placebo ($p < 0.05$)

DISCUSSION

In IUI and IVF, implantation failure may be the major cause of unsuccessful pregnancies. The suggestion that women with infertility may have an impaired uterine blood supply was first raised by Goswamy.¹⁷⁻¹⁹ Decline in endometrial receptivity, which is associated with a decrease in uterine perfusion, may

Table 4: Pregnancy distribution according to the value of the uterine score

Score	n	Pregnant	Pregnancy rate (%)
<i>M2-Tone</i>			
0-5	0	0	30.77%
6-10	0	0	
11-14	26	8	
<i>Placebo</i>			
0-5	0	0	16.66%
6-10	10	0	
11-14	14	4	

play an important role in the decrease of implantation rates. Pulsatility index (PI), which represents resistance to blood flow in the uterine artery was measured by color-image pulsed Doppler ultrasonography and it provided an indirect measure of endometrial receptivity.²⁰⁻²⁵ The PI of the uterine arteries has been known to diminish progressively during the luteal phase during which implantation occurs.²⁶ Estrogen is considered to play an important role as a modulator of uterine vascularity. It is reported that administration of estrogen improved uterine response in patients with poor uterine perfusion.^{17,26} Estrogen receptors have been identified on the walls of uterine arteries, and therefore, it is likely that the effect of estradiol on the uterine flow wave is a direct one.²⁷ Small amount of estrogen is enough to reduce substantially the uterine arterial PI.

The vascular changes are a reflection of the biochemical changes and can be studied by color doppler. In combination with 3-dimensional (3D) ultrasound, power Doppler provides a unique tool to quantify endometrial blood flow and, therefore, pregnancy rates.²⁸

In the M2-Tone supplemented patients, significant lower downstream impedance in uterine arteries was found. These data confirm that the decrease in peripheral impedance in the uterine vascular bed, reflected by a low PI, is a consequence of increased blood flow and tissue perfusion, which may improve uterine receptivity.^{21,25,26,29,30} A relationship has been found between vascularity and hormonal changes.³¹ The present study demonstrated an inverse correlation between the PI and endometrial thickness and estradiol levels in the M2-Tone group. The studies state that endometrial perfusion presents accurate noninvasive assay of uterine receptivity that may be used to predict implantation success rate.³² The reduced power Doppler signal at local level in the placebo group may be due to either suboptimal endometrial angiogenesis or dynamic vascular changes such as vasoconstriction or reduced vasodilation. Improvement in uterine score in M2-Tone group indicates that the drug improves the uterine health and endometrial receptivity, and thus the conditions conducive for successful implantation.

In this study, both the groups followed the same IUI protocol (Clomiphene citrate for ovulation and HCG for ovarian stimulation). Clomiphene citrate (CC) has been the most commonly used drug for ovulation induction in the past decades. Several hypotheses explain the mode of action of CC, but its mechanism and site of action still remain to be clarified. Though good ovulation rates (70%) can be achieved by CC, pregnancy rates are much lower, only about 20 to 40%. There is also a higher incidence of miscarriage. This contraindication in the ovulation and pregnancy rates can be attributed to the antiestrogenic effect of CC. Some studies have confirmed that the use of Clomiphene citrate decreases the uterine blood flow during the early luteal phase, a periimplantation stage.³³ Due to its long half-life and slow clearance; it persists in the body for a long time and adds to the accumulation of the antiestrogenic

effects. The antiestrogenic effect of CC can be overcome by administering estrogen concomitantly.³⁴

An alternative to CC is gonadotropins but they are associated with multiple pregnancy and ovarian hyperstimulation syndrome (OHSS).

M2-Tone is a herbomineral estrogenic formulation. It maintains the hormonal, nutritional and emotional balance in an infertile woman by virtue of synchrony between its various ingredients. M2-Tone encourages timely ovulation by balancing H-P-O axis and creates fertile intrauterine environment favorable for conception, nidation and fullterm gestation. The mild tranquilizing agents like *Nardostachys jatamansi*, *Asparagus racemosus* and *Cedrus deodara* restore emotional balance by relieving anxiety and tension. *Mesua ferrea* improves the endocrinal function and restores hormonal balance. *Nardostachys jatamansi* also has antispasmodic and anxiolytic activity. *Saraca indica* is reported to have a stimulating effect on the endometrial and ovarian tissue (providing a healthy development of ovarian follicles) and thus is a good uterine tonic.³⁵ *Cuminum cyminum* has a demonstrable estrogenic activity.³⁶ Kasis, an iron compound, which is one of the ingredients, supplements iron and thus improves the hemopoietic function. *Symplocos racemosa*,³⁷ and *Asparagus racemosus*³⁸ maintain the integrity of endometrium and may thus protect pregnancy, whereas *Zingiber officinale*,³⁹ *Mesua terra*⁴⁰ and *Shatavari*⁴¹ provide nutrition steadiness. Thus M2-Tone is a compact formulation catering to the problem of impaired endometrial receptivity and infertility.

The primary event initiating menstruation is tissue destruction and the loss of blood vessel integrity is one consequence of this. M2-Tone helps in rebuilding a healthy endometrium after menstruation for successful implantation and normal pregnancy. Some of the ingredients of M2-Tone *Asparagus racemosa*, *Cedrus deodara*, *Emblica officinalis*, *Mesua ferrea*, *Nardostachys jatamansi*, *Nelumbium speciosum*, *Saraca indica*, *Symplocos racemosa*, *Terminalia chebula* and *Zingiber officinale* are scientifically tested to have wound healing properties which aid in rebuilding of an endometrium competent enough for implantation and healthy pregnancy.^{42,43}

M2-Tone has been extensively used as an adjuvant therapy in all infertile cases. M2-Tone has also been presented in FIGO conference at Washington showing significant increase in pregnancy rates indicating improved blood flow in spiral arteries and uterine arteries following IUI use.

CONCLUSION

The study conducted at Malhotra Hospitals at Agra on 55 patients with infertility showed significant improvement in pregnancy rate when M2-Tone was given prior to an IUI protocol.

The improvement of the uterine artery blood flow in M2-Tone group was revealed as reduction in the pulsatility score by 1.13 as compared to 0.72 in placebo group. In M2-Tone group the endometrial thickness increased by 4.05 mm whereas in the placebo group it increased by 2.09 mm. The

upgrading in the uterine score in the M2-Tone group reflected as a significant pregnancy rate (30.77%) in M2-Tone group as against (16.66%) in placebo group.

M2-Tone was well-tolerated by patients and none of them reported any adverse effects. We recommend a treatment of M2-Tone prior to IUI procedures for increasing the chances of pregnancy through improving endometrial vasculature and suggest a multicentric trial with a bigger sample size for further evidence.

REFERENCES

1. Manners CV. Endometrial assessment in a group of infertile women on stimulated cycles for IVF: Immunohistochemical findings. *Hum Reprod* 1990;5:128-32.
2. Chiang CH, Hsieh TT, Chang MY, Shiau CS, Hou HC, Hsu SG, et al. Prediction of pregnancy rate of in vitro fertilization and embryo transfer in women aged 40 and over with basal uterine artery pulsatility index. *J Assist Reprod Genet* 2000;17:409-14.
3. Kdous M, Fadhlou A, Boubaker M, et al. Intrauterine insemination with conjoint semen. How to increase the success rate? *Tunis Med* Sep 2007;85(9):781-87.
4. Jinno M, Ozaki T, Iwashita M, et al. Measurement of endometrium tissue blood flow: A novel way to assess uterine receptivity for implantation. *Fertility and sterility* Dec 2001;76(6):1168-74.
5. Jose A, Riesewijk A, Dominguez F, et al. Determinant of Endometrial Receptivity. *Ann NY Acad Sci* 2004;1034:166-75.
6. Joglekar SN, et al. Estrogenic Activity of 'M2 Tone' An Indigenous Drug preparation. *Current Medical Practice*. Mar-82;26:3/108-11.
7. Parulekar VS, et al. Evaluation of M2-Tone syrup in functional menstrual disorders. Data on file.
8. Dubey P, et al. Clinical evaluation of M2-Tone in adolescent menstrual disorders. *Advances in Obstetrics and Gynecology*. Oct-Dec 2001;1(50):293-98.
9. Gupta I, et al. Menstrual disorders in adolescents. *Obs and Gynae. Today*. 2000 June;5(6):360-64.
10. Kotadawala P, et al. Evaluation of M2-Tone in menstrual disorders of adolescents and young adults. *Int J Gynecol Obstet India*. March-April 2004;7(2).
11. Urala MS, et al. Role of M2-Tone syrup in menstrual disturbance of reproductive age group women. *Obs and Gyne*. July 1999;4(7):469-71.
12. Tanksale VS, et al. M2-Tone Syrup in treatment of unexplained infertility in females. *Obs and Gynae* 1999;4(2):105-10.
13. Chandravati, et al. Clinical evaluation of M2-Tone in unexplained infertility. *Obstetrics and Gynecology Communications* 1999;1(6):50-54.
14. Malhotra N, et al. Endometrial scoring for prediction of implantation: Evaluation of drug therapy. *Obstetrics and Gynecology Communications* 2000;2(1):51-57.
15. Bhutani KK, et al. Effect of *Symplocos racemosa* Roxb. On gonadotropin release in immature female rats and ovarian histology. *Journal of Ethnopharmacology* 2004;94:197-200.
16. Salle B, Bied-Damon V, Benchaib M, et al. Preliminary report of an ultrasonography and colour Doppler uterine score to predict uterine receptivity in an in vitro fertilization program. *Human Reproduction* 1998;13(6):1669-73.
17. Goswamy RK, Williams G, Steptoe PC. Decreased uterine perfusion: A cause of infertility. *Hum Reprod* 1988;3:955-59.
18. Steer CV, Tan AL, Mason BA, Campbell S. Midluteal-phase vaginal color Doppler assessment of uterine artery impedance in a subfertile population. *Fertil Steril* 1994;61:53-58.
19. Groutz A, Wolman I, Jaffa A, et al. Influence of ovulation induction with human menopausal gonadotropin on uterine blood flow. Comparison of unexplained and mechanical infertility. *J Ultrasound Med* 1997;16:455-58.
20. Habara T, Nakatsuka M, Konishi H, et al. Elevated blood flow resistance in uterine arteries of women with unexplained recurrent pregnancy loss. *Hum Reprod* 2002;17(1):190-94.
21. Battaglia C, Larocca E, Lanzani A, Valentini M, Genazzani AR. Doppler ultrasound studies of the uterine arteries in spontaneous and IVF stimulated ovarian cycles. *Gynecol Endocrinol* 1990;4:245-50.
22. Bassil S, Magritte JP, Roth J, et al. Uterine vascularity during stimulation and its correlation with implantation in vitro fertilization. *Hum Reprod* 1995;10:1497-501.
23. Zaidi J, Campbell S, Pitroff R, et al. Endometrial thickness, morphology, vascular penetration and velocimetry in predicting implantation in an in vitro fertilization program. *Ultrasound Obstet Gynecol* 1995a;6:191-98.
24. Zaidi J, Jurkovic D, Campbell S, et al. Circadian variation in uterine artery blood flow indices during the follicular phase of the menstrual cycles. *Ultrasound Obstet Gynecol* 1995b;5:406-10.
25. Steer CV, Campbell S, Tan SL, Crayford T, Mills C, Mason BA, et al. The use of transvaginal color flow imaging after in vitro fertilization to identify optimum uterine conditions before embryo transfer. *Fertil Steril* 1992;57:372-76.
26. Goswamy RK, Steptoe PC. Doppler ultrasound studies of the uterine artery in spontaneous ovarian cycles. *Hum Reprod* 1988;3:721-26.
27. Perrot-Appianat M, Groyer-Picard MT, Garcia E, et al. Immunocytochemical demonstration of estrogen and progesterone receptors in muscle cells of uterine arteries in rabbits and humans. *Endocrinology* 1988;123:1511-19.
28. Panchal S, Nagori CB. Pre-hCG 3D and 3D power Doppler assessment of the follicle for improving pregnancy rates in intrauterine insemination cycles. *J Hum Reprod Sci* 2009;2(2):62-67.
29. Favre R, Bettahar K, Grnage G, et al. Predictive value of transvaginal uterine Doppler assessment in an in vitro fertilization program. *Ultrasound Obstet Gynecol* 1993;3:350-53.
30. Battaglia C, Artini PG, Giuliani S, et al. Colour Doppler changes and thromboxane production after ovarian stimulation with gonadotrophin releasing hormone agonist. *Hum Reprod* 1997;12:2477-82.
31. Bassil S, Magritte JP, Roth J, et al. Uterine vascularity during stimulation and its correlation with implantation in vitro fertilization. *Hum Reprod* 1995;10:1497-501.
32. Kupesic S, Kurjak A. Uterine and ovarian perfusion during the periovulatory period assessed by transvaginal color Doppler. *Fertil Steril* Sep 1993;60(3):439-43.
33. Hsu CC, Kuo HC, et al. Interference with uterine blood flow by Clomiphene citrate in women with unexplained infertility. *Obstet Gynecol* Dec 1995;86(6):917-21.

34. Selvaraj K, Selvaraj P. Comparison of clomiphene citrate and letrozole for ovulation induction and resultant pregnancy outcome. *J Obstet Gynecol Ind* 2004;54(6):579-82.
35. Srivastava GN, Bagchi GD, Srivastava AK. Ashoka: A potential ayurvedic drug. *Indian Drugs* 1987;24(11):496(a),507.
36. Malini T, Vanithakumari G. Estrogenic activity of Cuminum cyminum in rats. *Ind J Exp Biol* 1987;25:442-44.
37. Kiritkar KR, Basu BD. *Symplocos racemosa*. In: Blatter E, Caius JF, Mhaskar KS (Eds). *Indian Medicinal Plants* (2nd ed). 1990;2(2):1511-12.
38. Kiritkar KR, Basu BD. *Asparagus racemosus*. In: Blatter E, Caius JF, Mhaskar KS (Eds). *Indian Medicinal Plants* (2nd ed) 1990;4:2499-500.
39. Kiritkar KR, Basu BD. *Zingiber officinale*. In: Blatter E, Caius JF, Mhaskar KS (Eds). *Indian Medicinal Plants* (1st ed) 1990d;2435-38.
40. Kiritkar KR, Basu BD. *Mesua ferra*. In: Blatter E, Caius JF, Mhaskar KS (Eds). *Indian Medicinal Plants* 1990c;4(1):274-76.
41. Gaitonde BB, Jethmalani MH. Antioxytotic action of saponin isolated from, *Asparagus racemosus* (Wild Shatavari) on uterine muscle. *Arch Int Phann et de The M* 1969;179(1):121-29.
42. Salamonsen LA. Tissue injury and repair in the female human reproductive tract. *Reproduction* 2003;125:301-11.
43. Biswas TK, et al. Plant medicines of Indian origin for wound healing activity: A review. *Lower Extremity Wounds* 2003; 2(1);25-39.