ORIGINAL ARTICLE

Effect of endometrial thickness on pregnancy outcome after intracytoplasmic sperm injection

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Abstract

Objective: To identify cut-off value of endometrial thickness required for implantation of embryo after intracytoplasmic sperm injection.

Methods: The auasi-experimental study was conducted from July 2011 to June 2012 at an assisted reproductive clinic in Islamabad. Down-regulation of ovaries, controlled ovarian stimulation, oocyte pick-up, in vitro fertilisation, blastocyst transfer and confirmation of pregnancy with beta human chorionic gonadotropin more than 5mIU/ml. Patients were categorised into two groups on the basis of endometrial thickness <8mm and ≥8mm.On ovulation induction, before human chorionic gonadotropin injection, endometrial thickness was measured by trans-vaginal scan. Receiver operating curve was used to define groups on the basis of endometrial thickness cut-off value for pregnancy. The groups were compared in terms of the number of retrieved, mature and fertilised oocytes along with oocyte maturity, fertilisation and implantation rates by chi square test.

Results: There were 282 females; 116(41%) in Group A with endometrial thickness <8mm, and 166(59%) in Group B with endometrial thickness ≥8mm. In group A, 6(5%) and in Group B, 95(57.2%) patients had a positive pregnancy test. The number of mature, fertilised oocytes and cleaved embryos was significantly high in Group B (p=0.01; p=0.001; p=0.001respectively). Increase in endometrial thickness enhanced chances of oocyte maturity, fertilisation, cleavage and implantation (p<0.0001 each).

Conclusion: Endometrial thickness of 8mm was associated with a positive pregnancy outcome after intracytoplasmic sperm injection. Implantation of embryo was facilitated by better oocyte parameters, oocyte maturity, fertilisation and its cleavage in females who exhibited endometrial thickness above the cut-off value.

Keywords: Intracytoplasmic sperm injection, Ovulation induction, endometrial thickness, Implantation. (JPMA 65: 448; 2015)

Introduction

Humankind has attempted to alter its reproductive potential since time immemorial. There is a range of emotions that people experience when their expectations and beliefs are challenged by problems in achieving reproduction. Regardless of technical advances in assisted reproductive treatment (ART) procedures, success in terms of implantation rate (IR) has remained consistently low, averaging around 10-15%.[1,2] Infertility specialists make an effort to modify and refine ART to improve embryo implantation after in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI).

Embryo implantation is the outcome of successful intercellular interactions that form a link between encroaching embryo and receptive endometrium in a "window of implantation" at day 20-24 of a regular 28-day menstrual cycle.[3] In this event, a collaboration of factors makes the endometrium encroachable and amenable for approaching embryo.[4] The process is accompanied by vascular changes brought by timely interaction of ovarian steroid hormones supported by immune cells, cytokines, growth factors, chemokines and adhesion molecules.[5]

The changes in endometrium may be regarded as a reflection of endometrial proliferation under the influence of ovarian steroid hormones and cytokines.[6] Ultrasound measurement of endometrial thickness by trans-vaginal scan (TVS) is a simple and reproducible method to evaluate endometrial proliferation and has been studied as a possible indicator of uterine receptivity in fresh IVF cycles.[4,5,7,8]

The current study was planned to identify the thickness of endometrium required for implantation of embryo after ICSI.

Material and Methods

The quasi-experimental study was conducted from July 2011 to June 2012 at an assisted reproductive clinic in Islamabad. Using convenience sampling, females with age range of 20-40 years, duration of infertility more than 2 years, both ovaries

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present, menstrual cycle 28±7 days, body mass index (BMI) 18-30kg/m2, and basal serum levels of Follicle stimulating hormone (FSH) less than 10IU/mL were selected. Patients with uterine fibroids, metabolic disorders, short agonist and antagonist protocol were excluded. After initial consultation and screening of couples, long protocol with gonadotropin-releasing hormone agonist (GnRha) was given by daily injection DecaPeptyl from mid luteal phase of previous cycle. Controlled ovarian stimulation (COS) was provided with recombinant FSH (rFSH) injections for 12±2 days till ovulation induction (OI).On this day, endometrial thickness was measured by sonographers in the midsagittal plane by two-dimensional ultrasound with a 7.5MHz vaginal probe (Hitachi EUB 525; Hitachi, Tokyo Japan). Measurements were made at the thickest endometrial segment at the endometrial-myometrial interface in a 'frozen' midplane, longitudinal section of the uterus.[9] OI was made possible by intra-muscular (IM) injection of human chorionic gonadotropin (hCG) (Pregnyl 10,000 IU) 36 hours after which oocyte pick-up (OPU) was done under general anaesthesia.

Oocytes were microinjected with spermatozoa at right angles to the position of polar body under the microscope. Embryos were evaluated for cleavage till differentiation into blastocysts which were transferred five days after OPU. Pregnancy was declared by a positive pregnancy test by serum beta hCG measurement of specimens done 10days after embryo transfer (ET).[5] Patients were categorised into non-pregnant with β hCG<5mIU/ml and pregnant with β hCG>5mIU/ml. Pregnancy outcomes and associated rates were defined using standard art (SART) definitions.[10] Oocyte maturity rate was defined as total number of metaphase II oocytes/Total number of oocytes retrievedX100. Fertilisation rate was defined as the proportion of resulting in two pro-nuclei formation.[11,12] Mean implantation rate was the proportion of embryos transferred resulting in an intrauterine gestational sac that reflects the number of gestational sacs visualised on TVS divided by the number of embryos transferred.[11]

Data was entered into MS Excel and exported to SPSS 15for analysis. Clinical characteristics were summarised in terms of frequencies and percentages for qualitative variables (age group), mean standard deviation (SD) for continuous/quantitative variables. To find the best cut-off for the classification of pregnant group via endometrial thickness, Receiver operating curve (ROC) was constructed in Med Calc 12.5.0 software to depict probability of true-positive results (sensitivity) as a function of false-positive results (1-specificity). The adequacy of the test area under the curve (AUC; sensitivity/1-specificity) was set if was more than 0.5.

Results of groups were compared by chi square test. The different reproductive rates were transformed into <50% and >50%, and chi-square test was run to check association of oocytes maturity, oocytes retrieval and cleavage rate with outcome of pregnancy based on endometrial thickness group. Univariate logistic regression was executed to obtain odds ratio (OR) with 95% confidence interval (CI) to quantify this association.

Results

Out of 320 patients initially enrolled, 282(88%) completed the cycles till the transfer of embryos, and of them, 101(36%) were able to acquire clinical pregnancy (CP). There were 116(41%) women in Group A with endometrial thickness <8mm, and 166(59%) in Group B with endometrial thickness ≥8mm. In group A, 6(5%) and in Group B, 95(57.2%) patients had a positive pregnancy test (p<0.0001). Endometrial thickness correlated with ROC curve with AUC 87.5% (Figure-1). No significant difference was observed in demographic variables of the two groups. Mean duration of infertility in Group A and B was 7.18±4.07 and 7.012±3.60, and mean age was 32.17±4.61 and 32.02±3.73 years respectively. The average BMI in Group B and A was 24.29 to 24.26 kg/m^2 respectively. Mean FSH was 6.75±1.13 vs 6.64±1.06 in the two groups (p=0.375). The cycle patient characteristics in response to COS showed better response in Group B compared to Group A (Figure-2). Besides, females with oocyte maturity more than 50% became pregnant by acquiring endometrial thickness >8 mm (OR: 12.2; 95% CI: 2.7-54.4) (Table). Similarly, at

Table: Comparison of reproductive rates in study groups.

		Group A	Group B	P Value	OR	95% CI of OR
Oocyte Maturity Rate	< 50%	15 (88.2%)	2 (11.8%)	<0.0001	12.2	2.7- 54.4
	50% +	101(38.1%)	164(61.9%)			
Cleavage Rate	< 50%	16 (84.2%)	3 (15.8%)	<0.0001	8.7	2.5- 30.6
	50% +	100 (38%)	163 (62%)			
Implantation Rate	< 50%	108(52.2%)	99 (47.8%)	<0.0001	9.1	4.2 - 19.9
	50% +	8 (10.7%)	67 (89.3%)			
Fertilization Rate	< 50%	16 (84.2%)	3 (15.8%)	<0.0001	8.7	2.5 - 30.6
	50% +	100 (38%)	163 (62%)			

Values are numbers and percentages in parenthesis. Multiple logistic regressions on categorical variable of reproductive outcome rates and endometrial thickness.

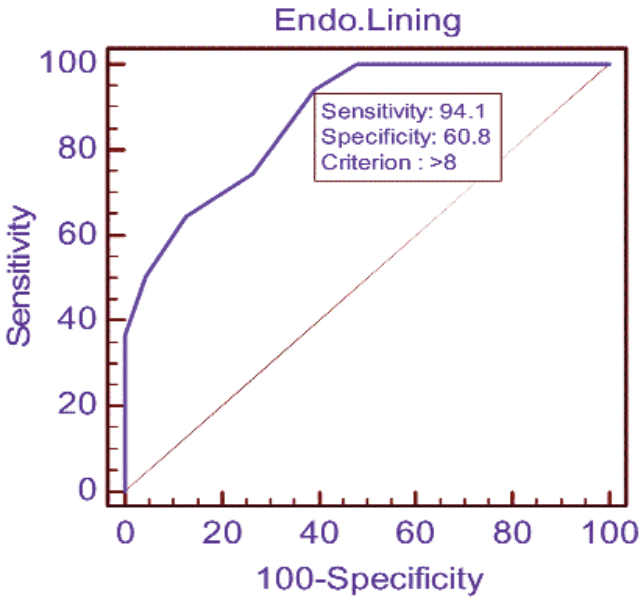

Figure-1: Receiver operating curve of endometrial thickness for clinical pregnancy measured on ovulation induction day.

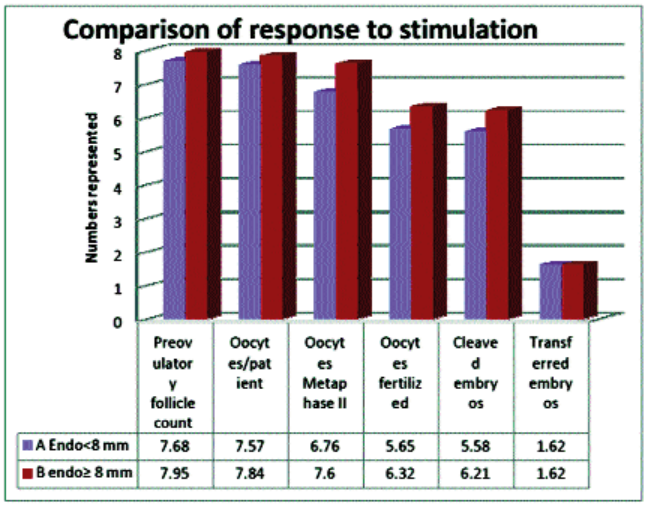

Figure-2: Comparison of ovarian response to stimulation in study groups.

cleavage stage, endometrial thickness was 8.7 times higher in females with cleavage rate greater than 50% (OR: 8.7; 95% CI: 2.5-30.6). Ahigh fertilisation and implantation rate was observed in patients with increased endometrial thickness.

Discussion

Endometrial receptivity accounts for a successful dialogue between progesterone primed endometrium and viable embryo in window of implantation for blastocyst implantation.[12,13] Receptivity is, therefore, the physiological characteristic of endometrium which permits blastocyst to attach, infiltrate, and bring about its decidualisation. Inadequate uterine receptivity accounts for more than 60% procedure failures which are credited to lack of optimal concentrations of hormones, collapse of pinopods over the micro villi and inadequate time synchronisation in the development of blastocysts and pinopods.[14] The 'gold standard' and ultimate proof of uterine receptivity is a successful outcome after ICSI which is manifested as conception.[9] The exact role of autocrine, paracrine and endocrine factors responsible for endometrial receptivity is yet to be explored.

Endometrial thickness, though predictive of the degree of endometrial maturation, there is no endometrial thickness value that confirms or excludes pregnancy in fresh ICSI cycles.[15] Several studies suggest a thickness less than 8mm with implantation failure in both fresh and frozen embryo transfer cycles.[4,7,8,15] Some authors have found association of favourable endometrial thickness with successful transfer of embryos in oocyte donation cycles,[16,17] while others failed to find such a correlation.[18,19] In medicated frozen-thawed embryo replacement (FER) cycles, an endometrial thickness of 9-14mm measured on the day of progesterone supplementation is associated with higher implantation and pregnancy rates compared with an endometrial thickness of 7-8mm.[5] An evaluation of endometrial pattern; multi-layered marked with three lines or non-multi-layered, however, was not found to be of any significance in the determination of endometrial receptivity.[9,14]

The importance of measuring endometrial thickness at around the time of embryo transfer helps to assure the presence of a minimal thickness to permit implantation of fertilised ovum.[8,20,21] Our study is unique in the sense that endometrial thickness was measured on OI day with an attempt to predict the likelihood of oocyte parameters, embryo quality and reproductive outcome rates. The impact of endometrial thickness on pregnancy outcome is supported by few investigators.[22,23] The controversies have been reported by some others.[20,24] In our group of patients, endometrial thickness of 8mm and above was associated with pregnancy, which is comparable to a study in which pregnancies occurred with endometrial thickness between 9 and 12mm.[14] Another study observed cut-off value of 9 and 10mm with higher pregnancy rates.[25]

It is quite evident that the success of ICSI depends on the quality of embryo as well as uterine receptivity. We found that patients in Group B with endometrial thickness more than 8mm had 50% enhanced oocyte maturity and fertilisation rates which increased probability of becoming pregnant. The thickness acquired was accompanied by increased chances of implantation to more than 50% and possibility of pregnancy to 4.2 times. These results are similar to a study in which

implantation potential was enhanced by an increased endometrial thickness.[5]

The results of our study emphasise the importance of estimation of endometrial thickness on OI day to have an insight about prediction of success after ICSI. The study, however, evaluated endometrial receptivity on the basis of thickness of endometrium only. The role of adhesion molecules, like integrins, anti-adhesion molecules, such as mucin 1, and detection of other immune endometrial markers required for endometrial receptivity were beyond the scope of our study. This, however, was the first study conducted on Pakistani women and emphasises the need of a cost-effective evaluation of endometrial thickness as an evidence of endometrial receptivity prior to giving infertile couples an expectation of the likely outcome.

Conclusion

Endometrial thickness of 8mm was associated with a positive outcome after ICSI detected by a rise in β hCG. These patients showed better oocyte parameters, embryo quality, oocyte maturity, fertilisation, cleavage and implantation rate. The importance of estimation of thickness on OI is important in the sense that if it is not ideal, some remedial action can be taken, such as postponing hCG administration and continuing ovarian stimulation, or freezing the embryos obtained for future transfer under better endometrial conditions. Endometrial thickness should be estimated on OI day before hCG administration in all patients undergoing ICSI treatment cycles.

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