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Correlation between Endometrial Thickness and IVF Outcome in an African Population

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Abstract

Background: The endometrium undergoes cyclic changes during the menstrual cycle in preparation for implantation. Successful implantation depends on a close interaction between the embryo and the receptive endometrium. The association between endometrial thickness and IVF outcome was investigated in this study.

Objective: To study the prognostic effect of endometrial thickness on outcome of *In Vitro* Fertilization (IVF) cycles

Method: A retrospective analysis of 267 IVF/ICSI cycles between 2009 and 2011 at Human reproductive research program of University of Benin Teaching Hospital was conducted. Cycle parameters were compared between pregnant and non-pregnant patients, main outcome measure being clinical pregnancy following IVF cycles. The endometrial thickness was measured on the day of human Chorionic Gonadotrophin (hCG) administration. The clinical pregnancy rates at each millimeter of endometrial thickness was evaluated to determine its predictive role

Results: Fifty-four among 267 cycles (20.2%) resulted in clinical pregnancy. The endometrial thickness on day of hCG administration was significantly higher in pregnant group compared to non-pregnant group [10.1 ± 1.7 mm versus 8.9 ± 2.0; p<0.0001]. The endometrial thickness cut-off value of at least 7 mm strongly correlated with clinical pregnancy.

Conclusion: Amongst other factors a thicker endometrial lining is associated with higher pregnancy rates. Effort should be made to institute protocols that will improve endometrial growth as a means of improving cycle outcome.

Keywords: Endometrial thickness; IVF cycles; Pregnancy; Infertility; Ultrasound

Introduction

Since the advent of assisted reproduction advances have been made in areas of ovarian stimulation regimen, method of assisted fertilization and improved culture techniques, yet the implantation potential of good quality embryo remains low. Several factors have been shown to influence the likelihood of implantation, one of which is endometrial receptivity [1]. In preparation for implantation the endometrium undergoes cyclic changes during the menstrual cycle. Successful implantation depends on a close interaction between the blastocyst and the receptive endometrium. Abnormalities of the endometrium such as asherman's syndrome that may prevent these changes have been associated with low implantation and high abortion rates [2].

Ultrasound evaluation of the endometrium during *In Vitro* Fertilization (IVF) cycles have been shown to be of clinical importance [3,4]. Albeit there is still no consensus on the correlation between endometrial characteristics and pregnancy rate demonstrated by several studies [5-14]. Some authors observed a greater probability of pregnancy once a threshold thickness is attained [5-10]. A threshold thickness of 7 mm and above was reported as positively predictive of pregnancy [15]. Noyes et al. and Rinaldi et al. [2,5] in their studies found significant pregnancy at endometrial thickness of 10 mm and above. Some other studies have failed to demonstrate any correlation between endometrial thickness and pregnancy [13,14]. Furthermore other workers have looked at the combined predictive role of endometrial thickness and pattern [16].

We sought to document our observations on the predictive value of endometrial thickness on outcome of IVF cycle. Ours is a developing nation, and with declining low socioeconomic state, there exist a huge financial burden on most of our clients. Thus in a low cost IVF setting like ours this study will impact on counseling and treatment planning in order to maximize potential for successful outcome.

Methodology

Setting

The Human Reproduction Research Programme [HRRP] is one of the four academic units of the department of obstetrics and gynaecology of the University of Benin Teaching Hospital, Benin City. It is a purpose built dedicated centre for infertility research and management in a public institution with provision for low cost IVF service.

Study population

Cycles of *In vitro* fertilization/Intracytoplasmic sperm injection (IVF/ICSI) conducted between 2008 and 2011 at the HRRP, were reviewed retrospectively. The Institutions local Review Board approval for the study was obtained. All fresh IVF or ICSI treatment cycles that used the long protocol (midluteal phase GnRH-agonist suppression) as the method of ovarian stimulation and reached oocyte pick up and embryo transfer within the study period were included, regardless of diagnosis, reproductive history, or insemination method.

Cycles using donor oocytes or cryopreserved embryos were excluded from this study. Other exclusion criteria included: the presence of known endometrial anomalies such as endometrial polyp or submucous myoma, and ovarian stimulation method other than the long protocol.

Protocol of controlled ovarian hyperstimulation (COH)

Pituitary down regulation [after synchronization of their menstrual cycles with sequential estrogen and progesterone to allow for batching] was with the GnRHa started in the mid-luteal phase of the preceding cycle and was continued through the follicular phase of the next cycle until the day of hCG administration. Controlled Ovarian Hyperstimulation (COH) with menopur was started on day 3 of the current cycle in an individual step-down protocol until a dominant follicle reached a diameter of 18 mm. Ovulation was triggered with 10,000 IU of hCG.

Follicular maturation and endometrial thickness were monitored by transvaginal ultrasound (Mindray°, model DP-6600 Shangai) examination at intervals, from day 6 to the day of hCG-administration. Endometrial thickness was measured in the midsagittal plane of the uterine body on the day of hCG administration. The largest thickness from one interface of the endometrial-myometrial junction to the other was measured.

Oocyte retrieval was performed via an ultrasound guided transvaginal approach 35 hours after hCG administration. Forty-eight hours after oocyte retrieval, embryos in transfer medium were implanted into the uterine cavity, and the recipient then rested in bed for 2-4 hours. Vaginal progesterone suppositories (400 mg/day) was started on the day of oocyte retrieval to provide luteal support and was continued daily until a pregnancy test was performed 15 days after embryo transfer. Clinical pregnancy was confirmed by identification of an intrauterine gestational sac by transvaginal ultrasound examination two weeks after positive pregnancy test.

For this study, the main outcome measure was clinical pregnancy. Cycle parameters were compared between pregnant and non pregnant patients.

Statistical analysis

Data were analyzed using SPSS version 16 software (SPSS Inc., Chicago, IL) and INSTAT stastical package as appropriate, all tests were two-tailed, and $p < 0.05$ was considered statistically significant. Continuous variables were presented as mean ± SD and were tested by the independent samples Student's T-test. Categorical data were expressed as numbers and compared using the Chi-square test. The predictive accuracy of endometrial thickness to predict the probability of pregnancy was assessed.

Results

A total of 267 IVF/ICSI cycles that met the inclusion criteria for this study were analysed. Overall 63 [23.6%] cycles resulted in a positive pregnancy test, of which 54 had pregnancy confirmation by identification of an intrauterine gestational sac on transvaginal ultrasound examination; clinical pregnancy rate was 20.2% and 9[3.4%] biochemical pregnancies. The biochemical pregnancies were regarded as negative pregnancy and excluded from the pregnant group in analysis. The age of patients ranged between 23 and 47, with mean age of 35.7 ± 5.0 and modal age of 38.

Table 1 shows a comparative analysis of cycles that eventuated in clinical pregnancy [pregnant] and those that did not [non pregnant]. The mean age between the groups showed that the pregnant group had significantly lower age 34.3 ± 4.3 vs 36.1 ± 5.1, (p = 0.02). Comparison of baseline characteristics between pregnant and non pregnant patient (Table 1) revealed no significant difference in characters such as type of infertility, previous children and duration of infertility. Although

majority had female factor infertility [46.8%], there was no statistical difference in causal factors among the two groups. There was no difference in the mean baseline FSH, the total dose of FSH used in ovarian stimulation and number of mature follicles. The number of eggs retrieved were higher in the pregnant group, (p = 0.0002). *In vitro* fertilization was more common compared to ICSI [84.3% vs 15.7%] but this was not significant statistically.

The endometrial thickness for the entire study group ranged between 5.3 mm and 18 mm with an average of 9.2 ± 2.0 mm. The mean endometrial thickness in the pregnant and non pregnant groups was 10.1 ± 1.7 mm and 8.9 ± 2.0 mm respectively and this difference was statistically significant (p = 0.0001). There was significant difference in terms of transfer technique and number of embryo's transferred between the two groups. The pregnant group significantly had better quality embryos transferred p = 0.0461. The process of transcervical transfer of embryos was graded as easy, moderate or difficult depending on the technical difficulties encountered. It was observed that there were more easy transfers for the pregnant group and no difficult transfer procedure recorded for this group in comparison to the non pregnant group and this was significant.

In Table 2, There was no pregnancy at endometrial thickness <7 mm whereas the non pregnant group had endometrial thickness <7 mm in 12% of the patients. The endometrial thickness value of ≥ 7 mm to <12 mm was significantly associated with pregnancy as there was no significant correlation with pregnancy when it is ≥ 12 mm or <7mm. Endometrial thickness cut-off value 10<ET ≤ 12 mm was highly predictive of positive cycle outcome, with improved pregnancy rates (28/88; 31%) at ≥ 10 mm endometrial thickness up to a high of 36% (18/50) at endometrial thickness ≥ 11 mm, p of 0.002; sensitivity 66.7%, specificity 15%, positive predictive value 16.6% and negative predictive value 64%.

Logistic regression analysis was evaluated to clinical pregnancy as dependent variable and variables which showed statistical association with pregnancy (shown in Table 1) included as independent variables. Those variables were patient's age, endometrial thickness, number of oocyte retrieved, quality of embryos and transfer difficulties. The endometrial thickness showed a significant independent influence on successful pregnancy (Table 3).

Discussion

Endometrial thickness has been identified to be a significant factor in embryo implantation in *in-vitro* fertilization cycles and by extension successful pregnancy. The predictive value of endometrial thickness in IVF cycle is strengthened by this study where we observed a significant independent association between endometrial thickness and rate of clinical pregnancy. The fact that the mean endometrial thickness was significantly higher for the pregnant group and an observed pregnancy rate highest at endometrial thickness of between 10-12 mm in this study, suggests that a thicker endometrial lining correlates strongly with pregnancy. An endometrial thickness above 7 mm was highly predictive of pregnancy. Our finding is similar to that observed in one of the largest study conducted to assess the combined effect of endometrial thickness and pattern on outcome of IVF cycles, where endometrial thickness had an independent positive correlation with pregnancy rate [16]. Several other investigators have reported similar findings [2,7,8]. However controversies still exist as regards getting a defined minimum cut off endometrial thickness predictive of successful pregnancy. Chen et al. [16] in their analysis found that the thinnest endometrial lining for successful ongoing pregnancy was 5.3 mm and further evaluation of clinical pregnancy rates according to each millimeter of endometrial

	Clinical Preg [n=54]	No Preg [n=213]	%	P value
Age				
Mean	34.3 ± 4.3	36.1 ± 5.1		0.02*
Type of infert				
1	8	59	67[25%]	
2	46	154	200[75%]	0.0545
Dura of infert				
[range 1-20]	6.3 ± 3.6	7.1 ± 4.3		0.1649
Prev children				
Yes	8	52	60[22%]	0.1476
No	46	161	207[78%]	
Cause of infert				
Female	26	99	125[46.8%]	
Male	9	33	42[15.7%]	
Combined	17	75	92[34.5%]	0.9503
Unexplained	2	6	8[3.0%]	
Basal fsh	9.3 ± 7.3	11.7 ± 9.5		0.095
[range 1-69]				
Total fsh dose	51.4 ± 14.5	60.4 ± 17.7		0.07*
[range 18-105]				
Follicle size>17mm	10.5 ± 4.8	11.8 ± 6.7		0.1063
Num of egg retrieved	8.2 ± 5.2	5.2 ± 4.4		0.0002*
[range 0-30]				
[Mean 5.8 ± 4.7]				
Num fertilized	4.5 ± 2.2	3.6 ± 2.3		0.120
Method				
Ivf	42	183	225[84%]	
Icsi	12	29	42[16%]	0.1473
Quality of embryo				
Excellent/good	40	128	168[62.92%]	0.046*
Good/reasonable	14	68	82[30.71%]	
Poor/reasonable	0	17	17[6.37%]	
Transfer				
Easy	30	144	174[65.4%]	
Moderate	24	58	82[30.8%]	0.022*
Difficult	0	10	10[3.8%]	
Endometrial thickness[mm]				
[mean 9.2 ± 2.0]	10.1 ± 1.7	8.9 ± 2.0		0.0001*
[range 5.3-18]				

*Statistically significant

Table 1: comparison of cycle parameters between the pregnant and non pregnant group.

thickness, showed an endometrial thickness threshold of 7 mm, below which pregnancy rates decreased rapidly. Reuter et al. reported that no patient conceived with endomterial thickness less than 8 mm [15]. In contrast other investigators have found a minimum thickness of 6 mm as an acceptable prerequisite for implantation [17,18]. Sundstorm [19] actually reported a successful pregnancy with an endometrial thickness as little as 4 mm but Dickey et al. [20] observed more frequent biochemical pregnancies with a thinner endometrium and an improved pregnancy rate with a thicker endometrium.

In this study, we found that an endometrial thickness of at least 7 mm increases the likelihood for pregnancy and pregnancy rates were higher when an endometrial thickness of at least 10<ET≤12 mm was achieved. Thus in an IVF cycle one should aim at achieving an

Endometrial thickness (mm)	Preg n=54	No preg n=213	Preg rate (%)	P value	OR	95% CI	Sen (%)	Spec (%)	PPV (%)	NPV (%)	LR
<7	31	0				[1.132-312.73]					
≥7	182	54	22.9	0.0013	18.814		14.5	100	100	22.8	
<8	6	72									
≥8	48	141	25.4	0.0007	0.2448	[0.100-0.599]	11.1	66.2	7.7	74.6	0.33
<9	16	113									
≥9	38	100	27.5	0.002	0.373	[0.196-0.709]	29.6	46.9	12.4	72.5	0.558
<10	26	153									
≥10	28	60	31.8	0.002	0.364	[0.197-0.671]	48.1	28.2	14.5	68.2	0.670
<11	36	181									
≥11	18	32	36.0	0.003	0.3536	[0.179-0.698]	66.7	15	16.6	64	0.784
<12	45	193									
≥12	9	20	31.0	0.142	0.518	[0.221-1.214]	83.3	9	18.9	69	0.92
<13	53	204									
≥13	1	9	10.0	0.692	2.338	[0.289-18.876]	98.1	4	20.6	90	1.025
<14	53	207									
≥14	1	6	14.3	1.000	1.536	[0.181-13.043]	98.1	2.8	20.4	85.7	1.01
<15	54	211									
≥15	0	2	0	1.000	1.288	[0.061-27.251]	100	0.94	20.4	100	1.009

Preg (pregnancy), OR;(odds ratio), sen(sensitivity), spec(specificity), PPV(positive predictive value), NPV(negative predictive value), CI(confidence interval), LR(likelihood ratio)

Table 2: Likelihood of clinical pregnancy at each millimeter of endometrial thickness.

Variable	B Logistic regression coefficient	S.E	Wald	P value	exp (B)	95% CI for exp B
Age	-0.007	0.038	0.032	0.857	0.993	0.923-1.069
Endometrial thickness	0.177	0.088	4.069	0.044*	1.194	1.005-1.418
Number of eggs retrieved	0.127	0.038	11.224	0.001*	1.136	1.054-1.224
Transfer difficulty	0.574	0.350	2.701	0.100	1.766	0.895-3.523
Embryo quality	20.572	9296.75	0.000	0.998	8.598	0.000

*Statistically significant

Table 3: logistic regression model.

endometrial thickness of at least 7 mm. Several other studies have demonstrated similar findings [5,6,20,21]. Paradoxically we observed a slight decline in pregnancy rate when thickness was above 13 mm, suggesting the possibility of a detrimental effect of very thick endometrial lining. Similar to our finding okohue et al. and Weissman et al. [10,14] while evaluating the effect of 'increased' endometrial thickness on IVF outcome reported lower implantation and pregnancy rates among women with an endometrial thickness >14 mm on the day of hCG administration. However, some authors have documented no adverse effect of markedly thickened endometrium on implantation, pregnancy or abortion rate [16,22,23]. We however, did not review the abortion and live birth rate in this study.

Despite the body of literature that suggests a positive correlation between a thicker endometrial lining and improved clinical pregnancy rates in IVF cycles, some studies have showed no significant association between endometrial thickness and rate of clinical pregnancy [9,11,12,24]. These lack of consensus in reports on the association between endometrial thickness and cycle outcome may be explained by differences in the method analysis. Some studies evaluated cut-off values [5,6,24], while others compared mean endometrial thickness [9,11] or compared endometrial thickness among percentile groups [24]. These different criteria might affect power to detect a small variation. In addition the day of measurement might also influence this conflict of results. Measurements could be made on the day of oocyte retrieval, the day of embryo transfer or on the day of hCG injection. Also various measurement methods (including outer edge to outer edge, or outer edge to inner edge) could further affect reported results. Furthermore other ultrasound parameters [such as endometrial pattern, volume and blood flow] could be utilized for evaluation of endometrial receptivity in addition to endometrial thickness.

Perhaps a review of research articles that used same methodology (in a bid to ensure uniformity) would resolve the present conflict of research findings. From the foregoing it is clear that allocating a minimum cut off endometrial thickness predictive of successful pregnancy may be difficult. Albeit a growing consensus of report tends towards accepting an endometrial thickness of at least 7 mm as being favourable.

An association of various cycle characteristics and treatment outcome has been evaluated since the introduction of assisted reproduction technologies. We observed that in addition to endometrial thickness, variables such as age, number of eggs retrieved, embryo quality and transfer difficulties also had significant impact on cycle outcome. Age has been strongly identified to influence IVF outcome

[25,26]. In this study pregnancy rate decreased as age increased, similar to the results of previous research [2,7,8]. This trend is in line with expected physiological reproductive potential where follicular response/maturation to controlled ovarian stimulation can be predicted as a function of age. Also we noticed a trend where in cycles that resulted in pregnancy, the patients were younger, had more oocytes retrieved, thicker endometrial lining, had better quality embryos transferred, and a less difficult transfer process. Similar to our findings, Kovacs et al. [2] had posited that although increased endometrial thickness is associated with higher pregnancy rates; neither the attainment of pregnancy nor pregnancy outcome was predicted by endometrial thickness alone. Endometrial thickness have been shown in the logistic regression model to significantly influence pregnancy independently [2,23]. This was corroborated in this study

In conclusion, we observed that overall, a thicker endometrial lining during IVF cycles correlated strongly with successful outcome. Also an endometrial thickness of at least 7 mm improved the probability of clinical pregnancy. We therefore advocate that in IVF centres (especially low cost centres like ours), effort should be made to institute protocols that will improve endometrial growth as a means of improving cycle outcome.

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