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Inherent Defect of the Endometrium Causes Implantation Failure in Assisted Reproductive Technique

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Abstract

Introduction: Implantation failure appears to be a significant factor in Assisted reproductive technique (ART) procedures. Even a mature endometrium may be non-receptive, preventing implantation or rejection of implanted embryo in early months of pregnancy, resulting in miscarriage or unexplained infertility with or without other associated factors.

Objective: To investigate causes of failed implantation in spite of uneventful Grade I embryo transfer in ART procedure.

Material and Method: 90 women aged range between 25-40 yr old who visited Department of Reproductive Medicine at Calcutta Fertility Mission, over a period of 24 months (January 2017 to December 2019), satisfying the inclusion criteria, were enrolled in this observational study.

Endometrial aspirate histopathology was done along with ∞5β3 integrin expression. They were treated with natural micronized progesterone (NMP) or oral dydrogesterone and results of endometrial changes were statistically analyzed.

Results: 28.89% and 31.11% of women were seen to have mid-secretory changes of the endometrium after being treated with NMP and dydrogesterone respectively. Integrins were expressed in only 59.26% of women with mid-secretory endometrium and 5.41% of early secretory endometrium, which was statistically significant (p value <0.001).

Conclusion: About 70% patients even after treatment with estrogen and progestin did not have adequate response in endometrial maturation. Not all patients with mid-secretory endometrium had integrin positive, when tested. NMP and oral Dydrogesterone have similar effect in endometrial maturation as well as in yielding successful pregnancy in some patients with previously failed In-vitro fertilisation embryo transfer (IVF-ET).

Keywords: In-Vitro Fertilisation; Implantation Failure; Midsecretory Endometrium; Integrin; Natural Micronized Progesterone; Dydrogesterone

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Introduction

With more and more popularization of Assisted reproductive techniques (ART) procedures, new interest has developed about implantation. It has been observed that signs of implantation are very important factor about the success or failure of ART. Large number of failures following the above procedure where fertilized egg transferred to hormonally prepared endometrial cavity fail to implant leading to loss of pregnancy. This may be due to embryonic defect, that is defective egg or sperm, or deficiency in the receptivity of the endometrium [1,2]. In our ART practice, In-vitro fertilization (IVF) failure cases have dictated us to find out the endometrial factor responsible for such implantation failure. It is difficult to detect the embryonal health in common set-up but when morphologically normal embryo fails to implant endometrial factor should be looked after by some simple procedure.

Material and Method

90 women aged range between 25-40 yr old who visited Department of Reproductive Medicine at Calcutta Fertility Mission, over a period of 36 months (January 2017 to December 2019). were enrolled in this observational study. The inclusion criteria were women who had undergone In-vitro fertilization and embryo transfer (IVF-ET) procedure at our clinic and did not have a viable pregnancy. Women with diagnosed Polycystic ovarian syndrome, endometriosis, uterine leiomyoma or septate or subseptate uterus, were excluded. Endometrial aspirate was taken on day 22-day 23 of next cycle and histopathology along with integrin were studied. On basis of the histopathology reports patients were given estradiol valerate (4mg) with natural micronized progesterone (NMP) (400mg per vagina/day) or oral dydrogesterone (20mg/day), for the next cycle, by random selection. They were re-assessed in a similar manner after the medications.

Ethical Consideration

The Ethical Committee of Calcutta Fertility Mission has given clearance for the retrospective study of a prospective database on 12/06/2019 (code: CFM/2019/038). Written informed consent has

been obtained from all women who participated in the study.

Statistical Analysis

Categorical variables are expressed as Number of patients and percentage of patients and compared across the groups using Pearson's Chi Square test for Independence of Attributes/ Fisher's Exact Test as appropriate. The statistical software SPSS version 20 has been used for the analysis. An alpha level of 5% has been taken, i.e. if any p value is less than 0.05 it has been considered as significant.

Results

90 patients in total had satisfied our inclusion criteria and were included in the current study. After IVF-ET failure these patients were subjected to histopathological examination of the endometrium without any exogenous medication. Based on the reports these patients were given estradiol valerate along with NMP or Dydrogesterone and were re-assessed with repeat histopathology and Integrin reports. 28.89% and 31.11% of women were seen to have mid-secretory changes of the endometrium after being treated with NMP and dydrogesterone respectively. 42.22% and 28.89% of them had shown early secretory changes or persistent non-secretory changes after treatment with NMP, respectively. Similarly, in women treated with dydrogesterone 40% had revealed early-secretory changes and 28.89% of women had non-secretory endometrium even after treatment with estrogen and progestin. 59.26% of women with mid-secretory endometrium and 5.41% of early secretory endometrium, tested positive for Integrin in the endometrial aspirate (p value<0.001) (Table 1 to Table 3).

Discussion

One of the major causes of implantation failure may be reduced endometrial receptivity. Balanced endometrial function and receptivity plays a key role in implantation process. Histological changes of whole uterine epithelium and stroma under hormonal influence along with cytokines and chemical changes make the endometrium receptive to the embryo as well as selective to the embryo as well. These endometrial changes are also influenced by embryo-endometrium crosstalk. Any disturbance in this harmony of endometrial decidualization leads to implantation failure, probably more than what we expect. A better understanding of endometrial receptivity is crucial to the creation and optimization of tests to assess the window of implantation in a clinical setting. A striking feature of the human endometrium is that the acquisition of a receptive phenotype in the mid-secretory phase of the cycle coincides with decidualization of the stromal compartment, irrespective of pregnancy [3-5]. Physiologically, the mid-secretory phase, is limited to approximately 48 hours and is characterized by up-regulation of several endometrial growth factors, cytokines adhesion molecules and over or under-expressed genes [6,7]. Most proposed endometrial receptivity markers are not specific to the uterus, and thus it would be difficult to determine whether their serum concentrations play a role in predicting successful implantation. Investigation of uterine flushings are therefore performed, however, has not been standardized yet, and the concentrations of components therein may vary [8]. Microarray analysis of endometrial tissue, genomic and proteomic analyses, mass spectroscopy and chromatography assessing levels of PGE2 and PGF2α, aspiration and assessment of secreted uterine fluids, called secretomics, during the secretory phase, that utilize high-throughput techniques with sophisticated large data analysis to generate detailed patterns of molecular and biochemical processes, has revolutionized our understanding of the receptive endometrium [9-12]. Previous literature has showed Endometrial Receptivity Array (ERA) is more accurate than histologic dating and is a completely reproducible method for the diagnosis of endometrial dating and receptivity status [13]. Though the implantation window lasts for about

Table 1: Age of women in groups.

		Group		Total	p Value	Significance
		NMP	Dydrogesterone			
Age	**25-30**	16(35.56)	15(33.33)	31(34.44)	0.965	Not Significant
	31-35	17(37.78)	17(37.78)	34(37.78)		
	36-40	12(26.67)	13(28.89)	25(27.78)		
Total		45(100)	45(100)	90(100)		

Data presented n (%); Pearson's Chi Square test for Independence of Attributes; NMP-natural micronized progesterone.

Table 2: Comparison of endometrial maturation in groups treated with NMP and Dydrogesterone.

		Group		Total	p Value	Significance
		NMP	Dydrogesterone			
Secretory	**Mid**	13(28.89)	14(31.11)	27(30)	0.968	Not Significant
	Early	19(42.22)	18(40)	37(41.11)		
	Non	13(28.89)	13(28.89)	26(28.89)		
Total		45(100)	45(100)	90(100)		

Data presented n (%); Pearson's Chi Square test for Independence of Attributes; NMP-natural micronized progesterone.

Table 3: Comparison of integrin expression in different phases of endometrium.

		Group		Total	p Value	Significance
		NMP	Dydrogesterone			
Secretory	**Mid**	11(40.74)	16(59.26)	27(100)	<0.001	Significant
	Early	35(94.59)	2(5.41)	37(100)		
	Non	26(100)	0(0)	26(100)		
Total		72(80)	18(20)	90(100)		

Data presented n (%); Fisher's Exact Test.

5 days with appearance of pinopodes, detected by electron microscopy, the endometrial receptivity remains best for about 48 hours. Hence, the former cannot be a clinical marker for implantation [14]. In a clinical setup histological assessment and measuring one or two standardized markers, become useful and reproducible. With this idea and in correlation with previous literature, endometrial aspirate on day 22 and day 23, was collected for histopathological examination and integrin (∞5β3) expression [15]. Expression of other special proteins, P27 and encyclin, were also tested in some cases with inconclusive results. In this study, mid-luteal phase histopathological examination and detection of integrin expression became the cornerstone for assessment of endometrial receptivity and implantation.

Several integrins have been identified as possible markers of uterine receptivity and have been noted to undergo alterations in the epithelium and decidua during implantation [15]. For clinicians detection of α5β3 expression, has become the hallmark of receptive endometrium. As has been seen in our present study expression of α5 β3 integrin in 59.26% of women with mid-secretory endometrium and 5.41% of early secretory endometrium, which was statistically significant (p value <0.001) (Table 3). No integrin was detected in women with non-secretory endometrium which indicates lack of integrin expression in the endometrium leads to infertility which correlates with previous literature [16,17]. Different phases of the endometrium have been seen to express different integrins, but in the mid-secretory phase α5β3 integrin expression has been seen to be the maximum, and hence has been considered as a hallmark in our study.

Although the luminal endometrial epithelium is the primary barrier in the implantation process, progesterone response in this cellular compartment that underpin the receptive phenotype are mediated by signals derived from the underlying stromal cells [18]. Progesterone induces secretory transformation and has immunomodulatory effect inhibiting tissue rejection, blocks the chemokines - transcription factor, leading to decreased prostaglandin synthesis & release positively regulating PIBF (Progesterone Induced Blocking Factor), on estrogen primed endometrium [19,20]. NMP has been seen to achieve mid-luteal 'Therapeutic' levels of Serum progesterone ≥ 14ng/ml as suggested by MHRA guideline [21]. Lotus I study has also demonstrated that a 20-fold lower dose of oral dydrogesterone (30 mg) is non-inferior to micronized vaginal progesterone (600 mg) for luteal phase support [22]. Similarly, in our study we have observed 28.89% and 31.11% of women with history of IVF-ET failure, had mid-secretory endometrium along with positive Integrin expression after being treated with estrogen and NMP or dydrogesterone. Both dydrogesterone and micronised progesterone were seen to be associated with similar rates of mid-secretory endometrial changes which further led to successful pregnancies in few (Table 2).

The problem lies with the fact that almost 70% of women in the present study, did not respond to estrogen and progesterone combination for endometrial maturation. Moreover, even in women with midsecretory endometrium only 59.26% had tested positive for integrin expression.

It has been demonstrated in the analysis of mid-secretory endometrial biopsies from patients with recurrent pregnancy loss (RPL) that there is an increased level of pro-inflammatory cytokine initially, a highly coordinated but transient inflammatory response that renders the endometrium receptive, before the endometrial stromal cells mount an anti-inflammatory response. Failure to constrain this pro-inflammatory response appeared to prolong the implantation window and was associated with RPL [23]. These can possibly explain the failed pregnancy or miscarriage, after embryo transfer, in mid-secretory phase in our study.

Conclusion

In our study although 30% women had mid-secretory endometrium after treatment, almost 70% of them in both groups had either early-secretory or persistent non-secretory endometrium. Integrin expression was also limited to 59.26%. Hence integrin expression should be considered as a better marker of endometrial receptivity rather than mid-secretory endometrium in cases of embryo transfer. Mid-secretory endometrium on histopathology cannot be considered as the sole factor for endometrial maturity and assessment.

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Conflict of Interest

There is no conflict of interest in between the authors involved in this study.

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