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Displacement of Window of Implantation in Cases of Unexplained Recurrent Implantation Failure as Detected by Endometrial Receptivity Array Testing

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Abstract: Patients with recurrent implantation failure had to be extensively studied to find way to achieve clinical pregnancy, it is well known that defects in the embryo or the endometrium or the alteration between both could be the reason. Exclusion of the possibilities of embryo abnormalities could help us to concentrate on the endometrial factors of failure. In this study we concentrated on endometrial receptivity displacement as a factor of implantation failure in patients with recurrent unexplained IVF failure. This is done through studying gene expression of endometrium in those cases to determine the receptivity timing and the window of implantation. Through retrospective study of 93 patients with recurrent implantation failure who underwent endometrial receptivity array testing, we found that the incidence of non-receptive endometrium in such cases was 45% 38 patients of 83 which is higher than other studies in the same field. Prevalence of pre receptive endometrium in those cases more than post receptive, Indicates the need to more exposure to Progesterone to achieve receptivity. Polycystic ovarian syndrome patients included in this study showed also high incidence of non-receptive endometrium10 out of 11patients (90.9%). In conclusion personalized embryo transfer according to ERA test could be useful to this category of patients. However larger studies are needed in the same group of patient

Keywords: Implantation, Implantation Failure, Array Test

1. Introduction

For pregnancy to happen an interaction between the endometrium and blastocyst in a complex process called implantation should be successful [1]. Recurrent implantation failure (RIF) is defined as inability to get clinical pregnancy after transferring high quality embryos in at least three in vitro fertilization cycles [2]. Multiple factors have been identified to increases the risk of implantation failure such as factors related to embryo [3], uterine anatomical, functional, and tubal factors [4].

human endometrium is the single uterine factor that directly affects the implantation, to understand this we should know that the endometrium is a dynamic tissue; it undergoes changes at multiple levels during the menstrual cycle in response to ovarian hormones and paracrine secretions resulting in proliferative and secretory phase of endometrium [5]. For the endometrium to accept the blastocyst and the implantation to happen it has to be receptive [2]. This endometrium receptivity happens in specific phase called window of implantation. During this time, the endometrium undergoes morphological, histological, biochemical, and genetic changes to become functionally receptive [5]. This is affected by the peak of progesterone level during the menstrual cycle. And it may last between 12 hours to 2 days which vary in length between patients [6]. Identification of the period of receptivity of the endometrium could be difficult. Endometrial receptivity could be assessed by morphological, histological, biochemical and genetic methods [7].

In IVF cycles morphological markers of the endometrium

could be detected using the ultrasound which include assessment endometrial thickness, character, and blood flow patterns [8, 9].

These noninvasive methods of endometrial assessment are not 100% accurate. Histological markers as epithelial pinopodes have been identified as a marker for endometrial receptivity but the presence of these pinopodes in post receptive endometrium have eliminated its value [4, 10]. Biochemical markers which have been identified in window of implantation as integrins, leukemia inhibitory factor, homeobox A10, mucin 1, calcitonin, and cyclo-oxygenase 2 have shown no relation to clinical application [11].

On the other hand studying the molecular markers that include studying gene expression in certain tissue and its relation to the function is called genomic signature [12].

Recently gene expression is the most established way available for evaluation of the endometrial receptivity [13, 14]. Extensive studies on endometrial gene expression has allowed a provisional recognition of the genomic signature of human endometrial receptivity [15], these studies have helped the scientists to differentiate the gene expression associated with each period of endometrial receptivity, that seven main groups of genes with a similar expression pattern throughout the cycle have been identified [16, 17]. Each of these groups had an expression peak in one of the seven sub phases (menstrual, early-proliferative, mid-proliferative, late-proliferative, early-secretory, mid-secretory, and late-secretory), early secretory phase is characterized by increased gene expression of gene associated metabolic activity and products related to cell metabolism like fatty acids, lipids, eicosanoids, and amino alcohols, it is also associated with increase in gene responsible of cell transport, and germ cell migration and all these are associated with inhibition of mitosis during this phase. But in the mid secretory phase there is up-regulation of most gene expression associated with high level of metabolic and secretory activity and up-regulation of genes involved in the activation of the immune response [17]. However during the late secretory phase down regulation of genes responsible of immune response-both cellular and humoral, blood coagulation, steroid synthesis, and prostaglandin metabolism is the dominant process [17]. As these activity define the window of implantation we can see that the early secretory phase is associated with pre-receptive endometrium, mid secretory associated with receptive endometrium and late secretory associated with post-receptive endometrium [18]. Testing for this activity in the endometrial tissue through highly sophisticated technique that extracts the genetic material (RNA) and analyzing hundreds of gene and comparing the gene expression to each period defines the endometrial receptivity in the given endometrial tissue [19]. It was believed that the window of implantation was the same in all women, But with gene expression testing of endometrial receptivity we realized that it differ from one lady to another and with the extend of studies it was shown that displacement of window of implantation could happen in one out of four RIF patients and in 20% of the general population [20].

In this study we are evaluating the mode of endometrium receptivity in unexplained recurrent implantation failure in morphologically normal endometrium using gene expression in endometrial tissue in the proposed period for window of implantation.

2. Methods

Our study is a retrospective cohort study carried on in large private IVF center in Abu Dhabi, in period between 2015 and 2020. Cases with unexplained recurrent implantation failure were included in the study.

2.1. Inclusion Criteria

primary infertility cases less than 43 years old, with more than 3 failed IVF cycles with embryo transfer of 2 or 3 good quality embryos in blastocyst stage, assessment of uterine cavity was done through hysteroscopy, at least one cycle of transfer of Euploid embryos. With at least one fresh and one frozen cycles. With all fertility work up including karyotyping of both partners, thrombophilia and antiphospholipid antibodies came normal, both tubes are free from hydrosalpenix through hystrosalpingography uterine cavity was assessed through hysteroscopy in all patients.

2.2. Exclusion Criteria

Cases above 43. Cases of secondary infertility. Cases with abnormal uterine cavity, cases with hydrosalpinex, cases with abnormal chromosomal embryos. Cases with thin endometrium less than 7 mm.

2.3. Hormonal Preparation

Patient included in the study were counselled and consented about the method and benefit of testing endometrial receptivity in their case. Patient started on estradiol valerate tablets 2 mg three times a day on their second or third day of menstrual cycle after ultrasound evaluation of endometrium to ensure endometrial shedding and normal ovaries, then after endometrium reaches 8 mm by transvaginal scan on day 8 of the start, progesterone vaginal suppositories started with dose of 400 mg twice a day for 5 days. If the endometrium did not reach the desired thickness, we added transdermal estrogen patch 100 mg that is to be changed every third day till endometrium reach 8 mm or more to start progesterone. Patients were called on day 6 of progesterone to collect endometrial sample. If patient had non receptive endometrium the biopsy to be repeated according to results of ERA test recommendation till we reach receptivity.

2.4. Endometrial Sample Collection

In sterile environment, patients were placed comfortably, then Cusco speculum introduced gently in the vagina and cervix exposed and cleaned. Then with endometrial Pipelle catheters gently introduced into endometrial cavity under ultrasonic guidance, the sample was collected and transferred to a tube containing 1.5 mL RNA stabilizing agent and shaken for a seconds, then kept at 4°C in

refrigerator for 4 h. the tissue obtained should be adequate and well-immersed in the fluid in the tube. If the tissue is too much, there is RNA degradation, and if too little, sufficient RNA is not available for extraction.

248 genes that are differentially expressed in the tissue using Next Generation Sequencing testing. This was adjusted to a computational predictor that can diagnose the personalized endometrial window of implantation of a given patient regardless of their endometrial histology. And the results interpreted as receptive endometrium, pre-receptive, post receptive, and non-valid sample. In case of non-receptive endometrium, the sampling to be repeated in a new cycle with sampling timing according to instruction sent by the laboratory.

2.5. Statistical Analysis

The most important parameter to be measured is the percentage of endometrial receptivity and the pre receptive and post receptive endometrium in relation to recurrent implantation failure and weather displacement of window of implantation had impact on implantation in cases of un explained recurrent implantation failure despite apparently normal endometrium.

Data were presented as the percentages, averages and mean in text, Table and graphs.

Age and body mass index presented in mean, previous cycles and frozen cycles presented in average. All data presented in actual numbers and percentages.

Chi square calculator was used to define the significance, Significance was set at $P<0.05$.

2.6. Approvals

This study was approved by research and ethical committee for health pulse network enabling to collect and analyze data available from patient's files and reports.

3. Results

The total cases of unexplained recurrent implantation failure were 93 case. 6 cases were excluded due to insufficient RNA, and 4 cases were with proliferative endometrium due to patient error.

Out of all the 83 patients 45 patients with receptive endometrium. (54.17%) in comparison patients with displaced endometrium were 38 (45.78%) p is 0.99 which is non-significant.

Table 1. *Number of Patients and Percentage with Receptive and Non-receptive Endometrium.*

Endometrium	Number Of Patients	Percentage %
Receptive	45	54, 17%
Non-receptive	38	45, 78%

Receptive endometrium had further classified to proper receptive which is the exact timing 33 patients (73%) and early receptive 5 patients (11%) and late receptive 7 patients (15.55%). patients in the early receptive group need more 12 hours to achieve proper receptivity but no need to another biopsy, and patients in the late receptive group needs to be 12 hours early with no other biopsy needed.

Table 2. *Classification of Receptive Endometrium.*

Classification	Number Of Patients	Percentage %
Early	5	11%
Late	7	15.55%
Proper Receptive	33	73%

Patients With post receptive endometrium were 13 patient (15.66%). Of the total patients included and (39.47%) of the non-receptive group. While patients with Pre receptive endometrium were 25 patients (30.12%) of the total patients included and 65.79% of the non-receptive group p is 0.75 which is non-significant.

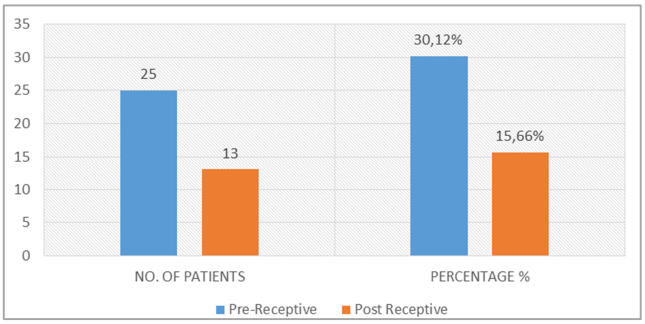

Figure 1. *Number of Patients And Percentage Of Receptive Endometrium.*

For patients with pre receptive endometrium we have to repeat the test after 24 hours in the next cycle (147±3) all came with receptive endometrium except two cases who we repeated again after another 24 hours (173±3) till it came receptive.

For patients with post receptive endometrium, we have to repeat the test 24 hours early in the next cycle (100±3 hours) all came receptive except one patient we have to repeat it again and it came receptive at 94±3 hours.

Table 3. *Patient Characteristics of Receptive and Non-Receptive Endometrium.*

Patient Criteria	Receptive	Non-Receptive
Age	35.8	36.5
BMI	28.1	27.5
PCO	1	10
Previous Cycles	5.1	4.3

In patients included in this study, the age ranges from 23-41 years old, with mean age 35.9 years.

Patient with receptive endometrium had mean age of 35.8, while non-receptive endometrium patient had mean age of 36.5.

Pre receptive endometrium had mean age 36.01 and post receptive endometrium had mean age 36.6.

The mean body mass index for all the patients was 27.9.

The mean body mass index for receptive endometrium group was 28.1, while the mean body mass index for non-receptive group was 27.5.

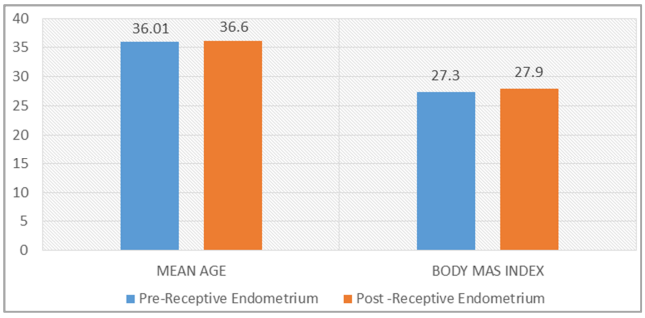

Figure 2. *Mean Age and Body Mass Index Of Receptive Endometrium.*

Table 4. *Mean Age and Body Mass Index of Non-Receptive Endometrium.*

Endometrium	Mean Age	Body Mass
Receptive Endometrium	35	28.1
Non-Receptive Endometrium	36	27.5

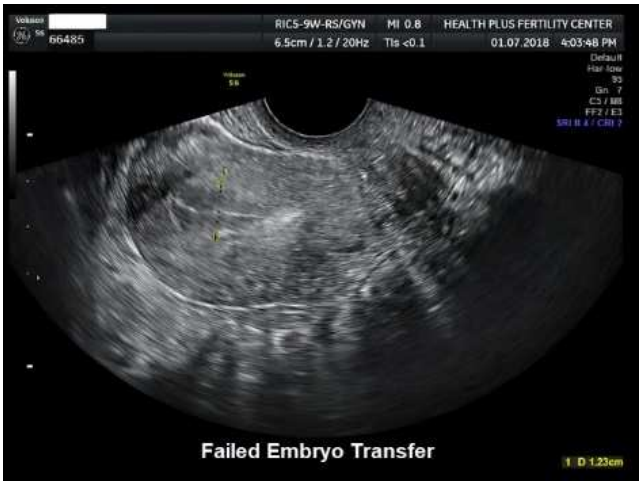

Figure 3. *Failed embryo transfer.*

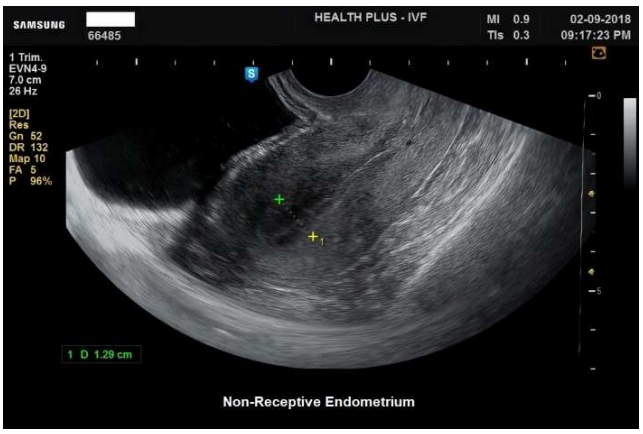

Figure 4. *Non-Receptive endometrium.*

The mean body mass index of pre-receptive group was 27.3, and the mean body mass index of post-receptive group was 27.9.

11 patients had PCOS, one came with receptive endometrium and 6 patients were with pre receptive endometrium and 4 with post-receptive endometrium.

The average numbers of embryo transfer cycles done before the era test ranges from 3 to 7 cycles per patient with mean of 4.67.

The average cycles before ERA test in the receptive group were 5.1 and the average cycles in the non-receptive group were 4.3.

The average number of cycles in the pre-receptive group was 4.5 with the average number of cycles in the post-receptive group was 4.1.

The time needed to prime endometrium ranged from 7-14 days to reach to the desired endometrial thickness.

9 patients took more than the 10 days to reach desired thickness which is > 7mm, all patients were in the pre-receptive group (36%). 6 of them were PCOS (66.7%).

The number of cycles with euploid embryo transfer ranged from 1 – 3 cycle per patients.

The patients had fresh and frozen cycles of transfer, the frozen cycles ranged from 1-4 cycles per patients.

Patients had day 3 and day 5 transfer, the range of day 5 cycle transfer was 1-5 cycles per patients.

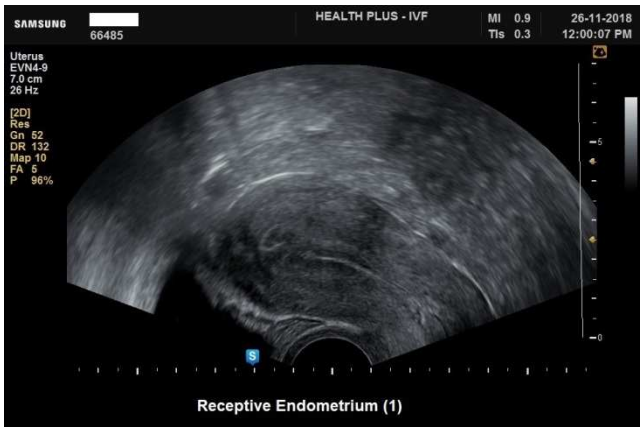

Figure 5. *Receptive endometrium.*

Figure 6. *Ultrasound showed pregnancy.*

4. Discussion

Recurrent implantation failure is a devastating situation for both patients and clinician especially with transfer of good grade euploid embryos, several studies had shown that endometrial receptivity have been displaced in around 25% of recurrent implantation failure cases but we can see from our study that unexplained recurrent implantation failure have

45,7% displaced window of implantation which is considered very high. This can be supported by study in 2019 by Hromadová L, et. Al., that found the non-receptive endometrium in patients with failed embryo transfer around 39% [19], and another study conducted in 2018 by Tan et al., found that patients with implantation failure after euploid embryo transfer have increased pregnancy rate after personalized transfer of euploid embryos more than standard transfer in cases with receptive endometrium [20]. On the other hand this percentage of displaced window of implantation was very high comparable to study conducted in 2019 by Jayesh A et al, That found the non-receptive endometrium occurred only in 17% of recurrent implantation failure cases [22], but we can see that they did not specify transferring euploid embryos so their study included wider spectrum of cases which the failure due to embryo aneuploidy could be the reason.

In our study we can see the pattern endometrium in non-receptive cases is more pre receptive (65, 79%) which indicates delayed response to progesterone in gene expression, this was supported by studies done in the same area by Jayesh A et, al in 2019 and Hashimoto et al,. 2017 [21, 22].

The average number of hours needed to reach the receptivity in pre receptive endometrium was more by 24 hours except in 2 patients (8%) who needed around 50 hours more to get receptive endometrium both patients were young age (29-30) years old and both were with PCOS.

PCOS patients presented with prolonged time needed to reach to accepted endometrial thickness and most of them fill in the non-receptive group 10 out of the 11 PCOS case with 6 of them in pre -receptive endometrium, which could be explained by abnormality recorded in patients with PCOS as a results of hyperinsulinemia, elevated free IGF-I and androgens, and obesity all likely contribute to endometrial dysfunction as could be explained by Kewei Shang, et. al. 2012 [23].

Post receptive endometrium was seen in 15, 66% of cases which indicated that the gene expression responds to progesterone in faster way than standard receptive endometrium which actually in agreement with studies in this field [18, 19].

Patient with post receptive endometrium needed less hours to reach receptivity around 24 hours early except one case who needed around 30 hours early to reach receptivity.

We can see that in cases with recurrent implantation failure especially with Euploid transfer the non-receptive endometrium is more prevalent than other cases with failed embryo transfer, personalized embryo transfer in these cases could help and achieve clinical pregnancy. Larger study of these group of cases is required.

5. Conclusion

The incidence of non-receptive endometrium increased in the patients with recurrent unexplained implantation failure. Those patients can benefit from personalized embryo transfer according to ERA test.

References

[1] Sheikhansari G, Pourmoghadam Z, Danaii S, Mehdizadeh A, Yousefi M. (2020), Etiology and management of recurrent implantation failure: A focus on intra-uterine PBMC-therapy for RIF. J Reprod Immunol. 2020 Mar 18; 139: 103121. doi: 10.1016/j.jri.2020.103121.

[2] Asher Bashiri, Katherine Ida Halper, and Raoul Orvieto, (2018) Recurrent Implantation Failure-update overview on etiology, diagnosis, treatment and future directions. Reprod Biol Endocrinol. Published online 2018 Dec 5. doi: 10.1186/s12958-018-0414-2 PMCID: PMC6282265. PMID: 30518389.

[3] P. De Sutter, R. Stadhouders, M. Dutré, J. Gerris, M. Dhont. (2012), Prevalence of chromosomal abnormalities and timing of karyotype analysis in patients with recurrent implantation failure (RIF) following assisted reproduction. Facts Views Vis Obgyn. 2012; 4 (1): 59–65.

[4] Quinn C, Ryan E, Claessens EA, Greenblatt E, Hawrylyshyn P, Cruickshank B, Hannam T, Dunk C, Casper RF. (2007), The presence of pinopodes in the human endometrium does not delineate the implantation window. Fertil Steril. 2007 May; 87 (5): 1015-21. doi: 10.1016/j.fertnstert.2006.08.101. Epub 2007 Jan 16. PMID: 17224147.

[5] Díaz-Gimeno P, Ruiz-Alonso M, Blesa D, Bosch N, Martínez-Conejero JA, Alamá P (2013),. The accuracy and reproducibility of the endometrial receptivity array is superior to histology as a diagnostic method for endometrial receptivity. Fertil Steril. 2013; 99: 508–17.

[6] Giudice LC., (1999), Potential biochemical markers of uterine receptivity. Hum Reprod. (1999) Dec; 14 Suppl2: 3-16. doi: 10.1093/humrep/14.suppl_2.3. PMID: 10690796 Review.

[7] Simón C, Martín JC, Pellicer A. Baillieres Paracrine regulators of implantation (2000), Best Pract Res Clin Obstet Gynaecol. (2000) Oct; 14 (5): 815-26. doi: 10.1053/beog.2000.0121. PMID: 11023802 Review.

[8] Pinar Cenksoy, Cem Ficicioglu, Gazi Yıldırım & Mert Yesiladali, (2013) Hysteroscopic findings in women with recurrent IVF failures and the effect of correction of hysteroscopic findings on subsequent pregnancy rates. Arch Gynecol Obstet. 2013; 287 (2): 357–360. doi: 10.1007/s00404-012-2627-5. [PubMed] [CrossRef] [Google Scholar].

[9] F Guerif, R Bidault, O Gasnier, M L Couet, O Gervereau, J Lansac, D Royere. (2004), Efficacy of blastocyst transfer after implantation failure. Reprod BioMed Online. 2004; 9 (6): 630–6. doi: 10.1016/S1472-6483(10)61773-7. [PubMed] [CrossRef] [Google Scholar].

[10] Craciunas L, Gallos I, Chu J, Bourne T, Quenby S, Brosens JJ, Coomarasamy A. (2015), Conventional and modern markers of endometrial receptivity: a systematic review and meta-analysis. Hum Reprod Update. 2019 Mar 1; 25 (2): 202-223. doi: 10.1093/humupd/dmy044. J Hum Reprod Sci. 2015 Jul-Sep; 8 (3): 121–129. doi: 10.4103/0974-1208.165153 PMCID: PMC4601169. PMID: 26538853.

[11] Zhang T, Li Z, Ren X, Huang B, Zhu G, Yang W, Jin L, (2018), Endometrial thickness as a predictor of the reproductive outcomes in fresh and frozen embryo transfer cycles: A retrospective cohort study of 1512 IVF cycles with morphologically good-quality blastocyst. Medicine (Baltimorre). 2018 Jan; 97 (4): e9689. doi: 10.1097/MD.0000000000009689.

[12] Nalini Mahajan, (2015). Endometrial receptivity array: Clinical application, J Hum Reprod Sci. 2015 Jul-Sep; 8 (3): 121–129.

[13] Quinn CE, Casper RF. Pinopodes. (2009), A questionable role in endometrial receptivity. Hum Reprod Update. 2009; 15: 229–36.

[14] Cavagna M, Mantese JC. (2003), Biomarkers of endometrial receptivity – A review. Placenta. 2003; 24 (Suppl B): S39–47.

[15] Díaz-Gimeno P, Ruíz-Alonso M, Blesa D, Simón C. 2014, Transcriptomics of the human endometrium. Int J Dev Biol. 2014; 58: 127–37.

[16] Ponnampalam AP, Weston GC, Trajstman AC, Susil B, Rogers PA (2004), Molecular classification of human endometrial cycle stages by transcriptional profiling. Mol Hum Reprod. 2004; 10: 879–93. [PubMed] [Google Scholar].

[17] S Talbi, A E Hamilton, K C Vo, S Tulac, M T Overgaard, C Dosiou, N Le Shay, C N Nezhat, R Kempson, B A Lessey, N R Nayak, L C Giudice (2006). Molecular phenotyping of human endometrium distinguishes menstrual cycle phases and underlying biological processes in normo-ovulatory women. Endocrinology. 2006; 147: 1097–121.

[18] Maria Ruiz-Alonso, David Blesa, Patricia Díaz-Gimeno, Eva Gómez, Manuel Fernández-Sánchez, Francisco Carranza, Joan Carrera, Felip Vilella, Antonio Pellicer, Carlos Simón (2013), The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. Fertil Steril. 2013; 100: 818–24.

[19] L Hromadová, I Tokareva, K Veselá, P Trávník, J Veselý (2019), Endometrial Receptivity Analysis - a tool to increase an implantation rate in assisted reproduction Ceska Gyneko. Spring 2019; 84 (3): 177-183, PMID: 31324106.

[20] Tan J, Kan A, Hitkari J, Taylor B, Tallon N, Warraich G, Yuzpe A, Nakhuda G. J Assist Reprod Genet. (2018). The role of the endometrial receptivity array (ERA) in patients who have failed euploid embryo transfers. Apr; 35 (4): 683-692. doi: 10.1007/s10815-017-1112-2. Epub 2018 Jan 11. PMID: 29327111.

[21] Tomoko Hashimoto, Masae Koizumi, Masakazu Doshida, Mayumi Toya, Eri Sagara, Nao Oka, Yukiko Nakajo, Nobuya Aono, Hideki Igarashi, Koichi Kyono (2017), Efficacy of the endometrial receptivity array for repeated implantation failure in Japan: A retrospective, two-centers study, Reprod Med Biol. 2017 Jun 27; 16 (3): 290-296. doi: 10.1002/rmb2.12041.e Collection 2017 Jul.

[22] Jayesh A Patel, Azadeh J Patel, Jwal M Banker, Sandeep I Shah, Manish R Banker (2019) Personalized Embryo Transfer Helps in Improving In vitro Fertilization/ICSI Outcomes in Patients with Recurrent Implantation Failure. J Hum Reprod Sci. 2019. PMID: 31007469.

[23] Kewei Shang, Xiao Jia, Jie Qiao, Jihong Kang, Youfei Guan (2012), Endometrial abnormality in women with polycystic ovary syndrome doi: 10.1177/1933719111430993. Epub 2012 Apr 24. Jul; 19 (7): 674-83 PMID: 22534323. DOI: 10.1177/1933719111430993.