**Author Query**

* **Kindly provide the intext citation for reference number 1**
* **Kindly modify/paraphrase the yellow highlighted text as the same is coming under high similarity to avoid any copyright issues in future**

**RECURRENT IMPLANTATION FAILURE**

**DR JAYESH AMIN, DR RIPAL PATEL**

**CHAPTER OUTLINE:**

**Introduction, Definition, Incidence, Causes of RIF, Investigations in case of RIF, How to proceed with a case of RIF- Counselling, Ovarian stimulation, Embryo transfer, Role of PGT, Role of ERA, Role of ImmunoTherapy, Role of intrauterine PRP, Surrogacy in RIF. Lab perspectives to improve the rate of qualitative blastocyst in RIF, Conclusion.**

**Introduction:**

Implantation failure refers to the term where embryo fails to reach the stage where it can be seen as a gestational sac on ultrasound. Again this refers to two kind of situations one in which there has been no recordable beta HCG and the other in which there was recorded beta HCG but it did not progress to the stage of gestational sac. In the present era where the field of ART has been so advanced, the unexplained recurrent implantation failure poses a great challenge.Still there are so much of debates regarding the ideal approach towards these cases that there is no definite evidence based proven approach about dealing with these cases.In this review we have tried to simplified the approach towards RIF cases and to reach to possible conclusion keeping in mind the current treatment options available.

**Definition:**

The term RIF is applicable to only those patients undergoing ART. There is no universally accepted definition of RIF. The following table depicts various definitions of RIF in the literature(1).

|  |  |
| --- | --- |
| **DEFINITIONS OF RIF IN THE LITERATURE** | |
| *Polanski et al., 2014b* | Absence of implantation after two consecutive cycles of fresh or frozen IVF embryo transfers with a cumulative number of transferred embryos of four or more cleavage-stage embryos or two or more blastocysts, all of good quality |
| *Coughlan et al., 2014* | Failure to achieve a clinical pregnancy after transfer of at least four good-quality embryos in a mini­mum of three fresh or frozen cycles in a woman under the age of 40 years |
| *El-Toukhy et al., 2016* | Two to four previous fresh or frozen IVF treatment cycles ending in an embryo transfer but no pregnancy |
| *Mariee et al., 2012* | Failure of three fresh IVF cycles or two fresh IVF and two frozen embryos transfer cycles |
| *Ledee et al., 2016* | Failure to have an ongoing pregnancy >10 weeks after at least six embryos were transferred on day 3 or day 5 in women aged <43 years |
| *Mitri et al., 2016* | Failure to have a clinical pregnancy after four or more blastocysts (fresh or frozen) after ruling out malformed uterine cavity, hydrosalpinx, abnormal karyotype or persistently thin endometrium in women aged 38 year or younger |
| *Kitaya et al., 2017* | Serial negative pregnancy tests following transfer of three or more morphologically good cleav­age-stage embryos and/or blastocysts |
| *Lensen et al., 2019* | Two previous implantation failures, no precision on number of embryos |
| *Olesen et al., 2019* | Implantation failure despite top-quality embryo or blastocyst transfer(s) |
| *Greco et al., 2014* | Three to nine previous implantation failures after IVF (mean 4.9) |
| *Huang, Wei & Li, 2017* | Two or more failed transfer of good quality embryos |
| *Koot et al., 2019* | Three failed IVF or ICSI treatments, each with at least one fresh good quality embryo per transfer, or failure to achieve pregnancy after transfer of 10 good quality embryos |

Regardless of the variability in definitions, the diagnosis of RIF is a difficult reality for many couples undergoing infertility treatment. There is as yet no universally accepted definition for RIF, despite many publications on this topic.

**Incidence:**

Due to variability in definition of RIF,there are no accurate data avalilable on exact incidence or prevalance of RIF. Biochemical pregnancy is reported to vary from 8 to 33% in general population which includes spontaneous conception also.

**Causes of RIF:**

The causes of RIF can be broadly divided into following three categories. Maternal factors, embryonic factors and paternal factors.

|  |  |
| --- | --- |
| **MATERNAL FACTORS** | |
| **MATERNAL AGE** | As age increases so the chances of aneuploidy leading to more implantation failures or abortions. |
| **BMI** | The pateints having class 1 , 2 and 3 type of obesity have highest rates of implantation failure. |
| **SMOKING** | Smoking disturbs the luteal phase by disturbing the function of corpus luteum thus leading to implantation failure. |
| **STRESS** | Elevated levels of cortisol due to stress leads to more chances of early abortions. |
| **UTERINE FACTORS & ENDOMETRIAL FACTORS** | Uterine fibroids, Endometrial polyps, Congenital uterine anomalies, intrauterine synechiae, Adenomyosis, Hydrosalpinx. |
| **THROMBOPHILIAS** | Antiphospholipid antibody syndrome |
| **CONNECTIVE TISSUE DISEASE** | Some of the disorders associated with RIF are Systemic Lupus Erythematosous, Rheumatoid Arthritis, Systemic Sclerosis, Primary Jogren’s Syndrome ,Inflammatory Myositis. |
| **IMMUNOLOGICAL FACTORS** | Desregulation of uterine NK cells, untreated Hypothyroidism , Cytokine Imbalance. |
| **EMBRYONIC FACTORS** | |
| **CHROMOSOMAL ABNORMALITIES** | Translocations,Mosaicism,Inversions and deletions are more common in RIF patients.The most common abnormality in RIF pateints is translocation.  Abnormalities in hatching process. |
| **PATERNAL FACTORS** | |
| Paternal factors associated with RIF are Varicocoel,Infections, Luecocytosspermia,Testicular torsion ,Testicular cancer,Systemic infections, Diabetes Sperm Chromosomal Aneuploidy, Sperm DNA fragmentation | |

**Investigations in case of RIF:**

There are ‘n’ number of investigations which are offered to cases of RIF worldwide. The following list of investigations is as per the recent Canadian Fertility and Andrology Society(2).

|  |  |
| --- | --- |
| **Level of Evidence** | |
| **Strong** | **Weak** |
| Hysteroscopy-routine use is not recommended | Parental Karyotyping |
| Thrombophilia testing is not recommended | DFI testing should not be routinely used.The use of DFI testing should be as such limited to research settings and counseling purpose. |
| Immunological testing-only for research purpose | Screening for chronic endometritis should not be routinely offered. |
| Endometrial Receptivity Array-limited to research settings |  |
| PGT-A:insufficient data to recommend for or against the case of RIF. |  |

Practically speaking, if the patient arrives after even one IVF failure in today’s era it is always advisable to counsel the patient to undergo PGT testing. The decision of ERA is a dynamic one. It depends on the number of PGT normal embryos one has.If there are less number of PGT normal embryos it is always beneficial to go for hysteroscopy and ERA testing and then to go for transfer.

**How to proceed in case of RIF?**

**COUNSELLING:**

The counseling should be stressed on following important points in previous one or more IVF failure:

-Possibility of Multiple ovum pick up in borderline ovarian reserve

-PGT A should be recommended

-Hysteroscopy as and when required

-The decision of ERA will be based on the number of PGT normal embryos.

-The possibility of donor oocytes in case of previous embryo arrest , consistently aneuploid embryos and consistently empty follicle syndrome.

-The possibility of surrogacy in idiopathic RIF.

**OVARIAN STIMULATION:**

Regarding ovarian stimulation if the history of previous stimulation is available , we can modify the dose accordingly.We will prefer to do genetic polymorphism testing of FSH and LH receptor before deciding the dose of stimulation.Otherwise we will give the standard dose of gonadotropins depending on wheather the patient belongs to normal ,hyper or hyporesponder group.We will prefer adding adjuvants in hyporesponders along with gonadotropins.For further details,kindly refer to the chapter on ovarian stimulation.

**EMBRYO TRANSFER:**

The current trend is towards blastocyst frozen embryo transfer in case of RIF patients.According to the current study published in 2019, the clinical pregnancy rate, implantation rate, and ongoing pregnancy rate were higher in the SBT group compared with the DET group (41.15% vs. 27.11%, *p* < 0.001; 41.15% vs. 19.28%, *p* < 0.001; 40.03% vs. 25.9%, *p* < 0.001)(3).They concluded that blastocyst stage transfer could be a preffered strategy in RIF patients.

**ROLE OF PGT:**

Performing preimplantation genetic screening for aneuploidy detection is believed to improve the implantation rates in RIF. Trophectoderm biopsy is preferred as risk of mosaicism is lower. Blastocyst analysis was associated with high pregnancy rates. Hence, the application of comprehensive chromosome screening is likely to assist a subset of RIF patients (those capable of producing blastocysts) in achieving pregnancies(4).

**ROLE OF ERA:**

The altered window of implantation(WOI) is one of the proposed cause of RIF. The ERA is the tool designed to find out this displaced WOI by studying the expression of genes of the proteins in the endometrium.According to one recent retrospective study published in 2017 that evaluated the role of ERA in RIF patients,41% of them were having non receptive ERA on which personalized emberyo transfer was performed. When analyzing implantation rates and pregnancy outcomes (live births plus ongoing second trimester pregnancies), both were notably higher in the receptive group compared to the non-receptive group even after pET(5).The decision of ERA should be a dynamic one depending on the number of PGT normal embryo one has.

**ROLE OF IMMUNOTHERAPY:**

There are several immunological factors responsible for RIF. But the results of immunotherapy are contradictory in RIF(6). The subcutaneous injection of G‑CSF can improve the outcome of patients with recurrent miscarriage(7) and the intrauterine infusion of G‑CSF in women with a thin endometrium increases the thickness of the endometrium and pregnancy rate. A thin endometrium is detrimental to ET and may cause RIF(8). The IVF success rate after the administration of IVIG increases in women with previous IVF failure, a high NK cell number, and/or an elevated Th1/ Th2 ratio(9). The optimal protocol for the administration of IVIG is undefined and the overall benefits of IVIG are still controversial. In 2006, Japanese scientists first reported that administration of autologous PBMCs(peripheral blood mononuclear cells) could significantly promote clinical pregnancy rate, implantation rate, and live birth rate in patients with repeated failure of IVF–ET(10).Then, Okitsu *et al(11)*demonstrated that only patients who had three or more implantation failures, the clinical pregnancy rate, and the implantation rate in the PBMC‑treated group were significantly higher than those in the nontreated group. It is better to recommend the immunotherapy after two or more implantation failures with euploid embryos.Still further research is required to design the exact protocol of immunotherapy in patient selection of RIF.

**ROLE OF INTRAUTERINE PRP INSTILLATION:**

Two RCTs investigated whether administration of intrauterine PRP could improve IVF outcomes in women with RIF. Pooling of results showed a significantly increased chance of clinical pregnancy in treated women(12,13). In RIF patients with good endometrium the role of intrauterine PRP is controversial.After PGT and ERA , the cases of implantation failures can have some beneficial effect of intrauterine PRP instillation but still more research is required to prove its exact role in idiopathic RIF.

**SURROGACY IN RIF:**

Factors responsible for RIF have important implication regarding treatment,however in many couples a definitive cause cannot be found(14).In those couples in whom no etiology is found after investigation of RIF and in whom empirical therapy was not effective ,surrogacy is a the only option(15).The role of intrauterine and intraarterial stem cell therapy is still experimental and more research is still required in this field.But it is always advisable to give the option of stem cell therapy for thin endometrium before going for surrogacy.Then it should be left on the patient to decide depending on their financial and psychological status.

**LAB PERSPECTIVES TO IMPROVE THE RATE OF QUALITATIVE BLASTOCYST IN RIF:**

At laboratory level , the factors that play an important role in failure of implantation are chromosomal abnormalities , damage to sperm DNA , zona hardening and inadequate culture conditions. Newer techniques like time lapse imaging , artificial intelligence(AI) software and metabolomics helps us to select a embryo with good implantation potential. AI software gives us two scores – viability score and genetic score. Based on the combination of these two scores , we can select the most appropriate embryo for transfer. If zona hardening is the problem , assisted hatching is the solution. The technique of ICSI and taking biopsy for PGT also are very important factors in the formation of the embryo.

**CONCLUSION:**

RIF with unknown cause significantly hampers IVF success.

It can be considered as an evolving field with a significant need for a well defined research. In the current generation of ART recipients and practitioners, the temptation to intervene after two or more failed embryo transfers is significant. The main goals in case of women presenting with RIF are getting a good quality genetically normal embryo and understanding the uterine receptivity. Empirical treatments should be offered only after aneuploidy cause of embryo has been ruled out and they should be evidence based.Counselling plays an important role in these cases which should be stressed on possibility of multiple ovum pick ups, embryo arrest, PGT-A and the role of ERA and other empirical treatments if all of these fails.

**REFERENCES:**

1.Maesawa Y, et al. History of biochemical pregnancy was associated with the subsequent reproductive failure among women with recurrent spontaneous abortion. Gynecol Endocrinol. 2015;31(4):306–8.

2.Tayla.S,Sony.S,Camille.S.Recurrent implantation failure in IVF: A Canadian Fertility and Andrology Society Clinical Practice Guideline. RBMO VOLUME 41 ISSUE 5 2020.

3.Xiaodong Z.et al.Frozen blastocyst embryo transfer vs. frozen cleavage-stage embryo transfer in couples with recurrent implantation failure: a cohort study. Hum Fertil (Camb). 2019.Jul 5;1-6 doi: 10.1080/14647273.2019.1633021.

4.Fragoului.E,Goodall.N,KatzJaffe.M,Schoolcraft.W.B,Munne.S,Wells.D.The role of aneuploidy in repeated implantation failure: implications for patient counseling and preimplantation geneticscreening.Volume90.https://doi.org/10.1016/j.fertnstert.2008.07.953.

5.Churchill.S.J,Bavan.B,Lathi.R.B.The prognostic value of endometrial receptivity array in recurrent implantation failure.| VOLUME 107, ISSUE 3, SUPPLEMENT , E7-E8, MARCH 01, 2017.

6.Cutting R, Morroll D, Roberts SA, Pickering S, Rutherford A, BFS and ACE, et al. Elective single embryo transfer: Guidelines for practice British Fertility Society and Association of Clinical Embryologists. Hum Fertil (Camb) 2008;11:131-46. doi: 10.1080/14647270802302629.

7.Scarpellini F, Sbracia M. Use of granulocyte colony-stimulating factor for the treatment of unexplained recurrent miscarriage: A randomized controlled trial. Hum Reprod 2009;24:2703-8. doi: 10.1002/jcb.21542.

8.Casper RF. It’s time to pay attention to the endometrium. Fertil Steril 2011;96:519-21. doi: 10.1016/j.fertnstert.2011.07.1096.

9.Winger EE, Reed JL, Ashoush S, El-Toukhy T, Ahuja S, Taranissi M, et al. Elevated preconception CD56+ 16+ and/or Th1:Th2 levels predictbenefit from IVIG therapy in subfertile women undergoing IVF. Am J Reprod Immunol 2011;66:394-403. doi: 10.1111/j.1600-0897.2011.01018.x.

10.Yoshioka S, Fujiwara H, Nakayama T, Kosaka K, Mori T, Fujii S, et al. Intrauterine administration of autologous peripheral blood mononuclear cells promotes implantation rates in patients with repeated failure of IVF-embryo transfer. Hum Reprod 2006;21:3290-4. doi: 10.1093/humrep/del312.

11.Okitsu O, Kiyokawa M, Oda T, Miyake K, Sato Y, Fujiwara H, et al. Intrauterine administration of autologous peripheral blood mononuclear cells increases clinical pregnancy rates in frozen/thawed embryo transfer cycles of patients with repeated implantation failure. J Reprod Immunol 2011;92:82-7. doi: 10.1016/j.jri.2011.07.001.

12.Nazari, L., Salehpour, S., Hosseini, M.S. & Hashemi Moghanjoughi, P. The effects of autologous platelet-rich plasma in repeated implantation failure: A randomized controlled trial. Hum. Fertil. (Camb). 4, 1–5 (2019).

13.Zamaniyan, M. et al. Effect of platelet-rich plasma on pregnancy outcomes in infertile women with recurrent implantation failure: A randomized controlled trial. Gynecol. Endocrinol. 2, 1–5 (2020).

14.Yanaihara A, Iwasaki S, Okai T. Causes and Treatment of Implantation Failure. Taiwan J Obstet Gynecol 2005;44:26-30.

15.Nastaran.A,Maryam.E,Behroz.A,Elham.R,Abass.A.Surrogacy as a good option for treatment of repeated implantation failure: a case series.Iran J Reprod Med Vol. 11. No. 1. pp: 77-80, January 2013.

16.Mausumi.D,Hananel.E.G.Holzer.Recurrent implantation failure:gamete and embryo factors.Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada.