Recurrent IVF failure

Dr sara saedi
Gynecologist
Fellowship of infertility

- IVF and embryo transfer has become an established and increasingly successful form of treatment for infertility.
- The last two decades have seen major advances in reproductive technologies, which along with improved data collection and documentation have increased public awareness of the availability and efficiency of assisted conception

- IVF and embryo transfer has become an established and increasingly successful form of treatment for infertility, yet significant numbers of couples discontinue treatment without achieving a live birth.

Discontinue treatment

- The number of couples who discontinue treatment and the reasons for discontinuation including live birth, lack of success, lack of funding, psychological stress, medical advice, physical discomfort, personal and other reasons.

- IVF failure is a problem for a couple in the singular but can be a tragedy in the plural.
- Recurrent IVF failure has multiple known causes
- The reason is there are several causes associated with lifestyle and other causes related to pre-existing conditions that have only a tenuous or no apparent connection to fertility

- Obesity
- Cigarette smoke
- Uterine anatomy
- BMI , thyroid dysfunction immune factors, the hereditary and acquired thrombophilias
- Embryo transfer technique on recurrent IVF failure.

- Despite advances in assisted reproductive technologies over the past four decades,
- patients remain who fail to achieve live births following multiple (IVF)/embryo transfer cycles.

Specific cause for RIF

- Patient age
- Genetic constitution of embryos
- Culture conditions
- Endometrial receptivity

(RIF)

- Recurrent implantation failure (RIF) is an characterized by failure to achieve pregnancy after repeated embryo transfers (ETs).
- Most currently used definitions are based on the number of previously failed ETs, while some also take into account other embryo-related factors, such as embryonic stage of development and embryo quality

- It is well established that female age is the strongest predictor of IVF success
- Another aspect of contemporary fertility practice is the increasing pre implantation genetic testing for aneuploidy (PGT-A), with euploid embryos having a better chance of producing an ongoing pregnancy, of producing an ongoing pregnancy

- A woman over 40 years of age is much less likely to achieve a successful pregnancy even after a significant number of ETs, compared to a woman in her early 30s, who may only require a small number of transfers of comparable embryos to achieve a similar cumulative pregnancy rate.
- Furthermore, availability of PGT-A tested euploid embryos further influences cumulative pregnancy rate computation

- Embryonic euploidy is one of the greatest determinants of successful conception
- However less than 60% euploid embryos result in an ongoing pregnancy
- Normal endometrium is an important part of the implantation process

- It is difficult to account for the possible detrimental effects of laboratory processes, such as embryo biopsy and cryopreservation, in these outcomes.
- The diverse aetiologies including maternal, male and embryo factors and incomplete understanding of RIF provide significant diagnostic and therapeutic challenges to patients, researchers and clinicians.

- The probability of obtaining euploid embryos decreases with age, and this decrease also accelerates over the age of 40 years.

Maternal age remains the single most important variable in predicting successful implantation.

- Female age is not only associated with an increase in embryo aneuploidy ,but also parallels a decline in ovarian reserve and response to gonadotropin stimulation.
- Although ovarian reserve testing can help anticipate response to gonadotropin stimulation and may correlate with success rates, it falls short of predicting cycle outcome.

- Embryos have a high attrition rate in the laboratory and following implantation, owing mostly to genetic abnormalities.
- The introduction of closed incubator systems utilizing continuous time-lapse monitoring of embryos in stable, may decrease the impact of an artificial laboratory environment on embryos.

RIF

- Defined by the absence of implantation after three or more transfers of high-quality embryos
- Or after transfer of >10 high-quality embryos in multiple cycles.

PARENTAL GENETICS

- Translocations represent a variety of rearrangements between non-homologous chromosomes.
- They can be reciprocal, whereby two non-homologous chromosomes exchange segments, or Robertsonian, when two acrocentric chromosomes break at their centromere to fuse as a single, large chromosome with the loss of the two short arms.

- Small portion of couples with RIF will have abnormal karyotypes, the incidence of parental chromosomal abnormalities is reported to be 2.5%
- By comparison, the incidence of translocation carriers in couples with recurrent pregnancy loss was found to be 4.7%.

Parental karyotypes

- Parental karyotypes should be performed in couples with RIF.
- Couples with known translocations should be carefully counseled with providing advice pre implantation genetic diagnosis (PGD).

BLASTOCYST CULTURE

- Identification of embryos with a higher implantation potential is key to improving the efficiency of IVF.
- Cleavage-stage embryo development is controlled by maternal RNA transcripts until the four- to eight-cell stage RNA transcripts until the four- to eight-cell stage
- Activation of the embryonic genome begins on day 3 of development and continues to the blastocyst stage, a stage that confers a higher implantation potential than cleavage-stage embryos.

- The first culture stage (pronuclear stage to compaction) consists of non-essential amino acids, ethylene diamine- tetra acetic acid (EDTA), and pyruvate and a reduced glucose concentration.
- The second culture stage (compaction to blastocyst) adds essential amino acids, removes EDTA, reduces the pyruvate concentration, and increases the glucose concentration to meet the increased energy demands of the embryo during rapid cell division.
- Sequential culture media thus facilitates the selection of embryos most suitable for transfer.
- Recently, global culture media have been developed that are also suitable for efficient blastocyst development.

- While a high of embryos fail to form blastocysts due to genetic aneuploidy, a subset of embryos arrest at the cleavage stage due to suboptimal culture conditions.
- Earlier studies suggesting a higher implantation rate with blastocyst transfers may have used select patient populations with good prognoses for implantation

- The ideal candidates for blastocyst transfer are high ovarian responders to gonadotropins who create excess embryos, allowing one to select the best available blastocysts to enhance implantation rates.
- Poor responders with limited numbers of embryos are not good candidates for prolonged culture conditions as they may arrest at cleavage stages prior to transfer.
- Most patients with RIF fall into the second category where prolonged culture of a small number of embryos appears to offer no significant advantages.
- For those who are high responders, prolonged culture conditions may improve the implantation rate and clinical success.

EMBRYO GENETICS

- A direct correlation between female age and oocyte aneuploidy exists, with the steepest rise in aneuploidy occurring in the late 30s and early 40s.
- (FISH) to diagnose numeric abnormalities of X, Y, 18, 13, and 21, noted aneuploidy rates for these five chromosomes to be 37% for women aged 40–47 years.
- Comparative genomic hybridization (CGH) for all 24 chromosomes, aneuploidy rates have been found to range from 58% at 40 years of age to 100% at 47 years of age
- This underscores the importance of female age in predicting the implantation potential of embryos.

- The purpose of these techniques is to increase implantation and live birth rates, as well as to reduce the risk for spontaneous abortion and associated chromosomal abnormalities discovered at birth.
- Biopsy and FISH analysis of cleavage-stage embryos has largely been abandoned, owing to a high rate of mosaicism at day 3 and self-correction capabilities that led to mis diagnosis of aneuploidy.

- As embryo culture techniques and the efficiency of reaching the blastocyst stage *in vitro* have improved
- PGS by trophectoderm biopsy has witnessed a resurgence.
- CGH is a molecular technique that allows for an entire genome to be scanned for variations in DNA copy number.
- Total genomic DNA is isolated and compared to a reference cell, differentially labeled, and hybridized, allowing for comparison of all 24 chromosomes in a single interphase cell
- Specific trials have looked at the outcomes benefiet of PGD for aneuploidy screening in RIF patients.

- Day-3 blastomeres were tested by FISH for chromosomes 13, 16, 18, 21, 22, X, and Y.
- A higher rate of aneuploidy (67.4% vs. 36.3%) was observed in patients with RIF compared to age-matched controls. The pregnancy rates between the RIF group and controls were comparable(34% vs 33%)
- In a similar study, Gianaroli et al. found that the percentage of chromosomally abnormal embryos increased proportionally with the number of prior IVF failures
- CGH analysis of blastocysts failed to demonstrate a difference in 43 RIF patients compared to 45 infertile, good-prognosis patients (aneuploidy 53.8% vs. 48.2%, respectively)
- Thus, further studies are needed to assess whether embryonic aneuploidy is responsible for RIF in the absence of parental chromosomal abnormalities.

- Prospective data examining whether PGS benefits patients with RIF are mixed, and largely rely on data from older FISH technologies.
- Gianaroli et al. reported a significant improvement in implantation (28.0% vs. 11.9%) in a small prospective study when day-3 biopsy and FISH analysis were employed
- Conversely, other studies have suggested a negative impact of PGS for RIF patients, with significantly lower live birth rates after biopsy (21%) as compared to no biopsy (39%)
- Indeed, the negative impact of PGS in individuals with RIF was confirmed by a recent meta-analysis

- Trophectoderm biopsy has several advantages over blastomere biopsy, including greater developmental resiliency, less mosaicism, and the ability to analyze multiple cells,
- Blastocyst-based PGS has been suggested to improve the efficiency of IVF in older individuals (>40 years of age) undergoing IVF

- Another retrospective study claimed a benefit in implantations and live births when PGS was used in women aged 40–43 years with multiple prior IVF failures, with a live birth rate for PGS
- Frozen embryo transfer with PGS (45.5%)
- Fresh transfer without PGS (15.8%)
- Frozen transfer of non-PGS embryos (19.0%)

- The benefit of blastomer biopsy with 24-chromosome PGS is to reduce the incidence of viable trisomies and spontaneous pregnancy loss, both of which affect older patients (>38 years of age) disproportionately.
- The benefit of PGS for young patients, and specifically young patients with RIF is less established, and is an area deserving of careful study.
- Biopsy and analysis does not, however, intrinsically increase the implantation potential of any given euploid embryo, and indeed adds cost, invasiveness, and the potential for discarding a normal embryo

SPERM GENETICS

- Sperm concentration, motility, and morphological assessment are relatively poor predictors of conception with assisted reproductive technology.

DFI

- Recently, tests of sperm DNA integrity have been increasingly used for evaluating spermatozoa in conjunction with semen analyses.
- Sperm DNA damage is correlated with poor reproductive outcome, including increased pregnancy loss and chromosomal aneuploidy
- Damage of sperm DNA has also been associated with poor development of embryos, increase in miscarriage risk in spontaneous pregnancies, its role in patients with RIF remains uncertain

- In RIF couples high DNA fragmentation has been documented, the use of testicular-retrieved spermatozoa has been suggested
- Greco et al. studied 18 couples with at least two unsuccessful IVF/ ICSI attempts where male partners had ejaculated spermatozoa with >15% DNA damage by TUNEL assay
- DFI in their testicular sperm (4.8%) was lower as compared to ejaculated specimens from the same individuals (23.6%),
- 8 clinical pregnancies (44.4% clinical pregnancy rate) when testicular sperm was used for ICSI.

- Along with sperm DNA fragmentation, sperm aneuploidy may also play a role in RIF.
- Men with abnormal semen parameters have been noted to have increased aneuploidy rates in randomly collected semen samples.
- Men with lower sperm aneuploidy demonstrated higher implantation (34% vs. 13%) and pregnancy (75% vs. 34%) rates, as well as lower miscarriage rates (38% vs. 11%)

UTERINE PATHOLOGY

- An evaluation of the uterine cavity is warranted in patients who have experienced repeated IVF failures after transfer of high-quality embryos.
- Fibroids, polyps, intrauterine adhesions, chronic endometritis, or Mullerian anomalies have all been implicated in RIF.
- The incidence of previously unrecognized intrauterine pathology in individuals with RIF may be elevated; in fact, it was as high as 25%–50%

- Fibroids adversely affect implantation: mechanical obstruction of tubal ostia, chronic intracavitary inflammation, and increased uterine contractility
- It is generally accepted that subserous myomas do not adversely affect pregnancy or live birth rates, and thus removal is rarely warranted.
- It is equally agreed upon that submucous myomas decrease pregnancy rates and increase the incidence of miscarriage.
- While the benefits of submucous myoma resection are clear, the benefit of myomectomy
- Intramural myomas located outside of the endometrial cavity is more controversial

- Endometrial polyps can also diminish implantation rates
- Multiple retrospective studies have reported improved spontaneous conception rates when endometrial polyps are resected
- A randomized controlled trial of 215 infertile women with polyps that compared a group undergoing polypectomy with those undergoing a diagnostic hysteroscopy without intervention revealed that pregnancy was 2.1-times more likely after polyp resection
- Previous uterine instrumentation, especially those complicated with pelvic infection, should prompt investigation for intrauterine adhesions.

- Chronic endometritis should be excluded in patients with RIF without apparent cause.
- Several researchers have implicated endometrial inflammation as a potential etiology of RIF
- The incidence of chronic endometritis based on histological evidence of plasma cells has been estimated to be as high as 30.3% in patients with RIF.
- Lower implantation rates have been noted in patients with evidence of endometritis

- Patients cultured positive for Gram-negative bacteria were treated with ciprofloxacin for 10 days
- Gram-positive bacteria were treated with an eight-day course of amoxicillin and clavulanate.
- Patients with endometritis in the absence of positive cultures were treated with a single dose of intramuscular ceftriaxone followed by a 14-day course of oral doxycycline and metronidazole.
- Live birth rates were 60.8% in the patients in whom endometritis was successfully treated versus 13.3% for those in whom evidence of endometritis persisted after treatment.

- Adenomyosis occurs when endometrial glandular cells invade the uterine myometrium.
- (MRI) with contrast is the best diagnostic imaging modality.
- Though adenomyosis has been implicated as having a significant negative affect on female fertility
- Limited reports in patients with RIF have suggested successful treatments with ultra-long pituitary gonadotropin-releasing hormone agonist down-regulation prior to IVF

- HSG, saline infusion sonography, three-dimensional ultrasonography, MRI, and hysteroscopy are all available for evaluation of uterine architecture and the endometrial cavity.
- Two prospective, randomized controlled trials have suggested a benefit of performing routine hysteroscopy in all patients with RIF, with reported detection rates of abnormal findings ranging between 25% and 50%
- HSG provides data on the endometrial cavity as well providing information on the status of the fallopian tubes (i.e., hydrosalpinges);
- A prospective study comparing vaginal sonogram, SHG, and diagnostic hysteroscopy concluded that hysteroscopy offered a more thorough detection of intra cavitary lesions than SHG and transvaginal ultrasound

TUBAL PATHOLOGY

- The mechanisms hydrosalpinges adversely affect reproduction are potentially multifactorial: accumulated tubal fluid may exert a direct embryo toxic effect, may act to mechanically flush an embryo from the uterus, or may adversely alter endometrial receptivity
- Evidence suggests that live birth rates in patients with hydrosalpinges undergoing IVF are reduced

- Leukemia inhibitory factor (LIF), an endometrial cytokine, was restored to normal following salpingectomy Avb3 integrin expression is similarly restored following salpingectomy
- Pregnancy rates of 23.9% and live birth rates of 16.3% in IVF patients in whom hydrosalpinges were left untreated as compared to 36.6% and 28.6%, respectively, when salpingectomy was performed prior to IVF).

Assisted hatching

- Zona hardening and subsequent lower rates of hatching and blastocyst expansion than occurs *in vivo* Cleaved embryos with reduced zona thickness have higher implantation rates than those with thick zonae.
- Thus, it was suggested artificially opening or thinning of the zona could facilitate the hatching process
- A variety of techniques have subsequently been developed to aid in the hatching process, including mechanical partial zona dissection, chemical drilling using acid Tyrode's solution, enzymatic thinning, laser-assisted hatching
- It has been proposed that such techniques not only aid in mechanical hatching, but also could enhance transport of nutrients from incubating media by allowing for a two-way exchange of metabolites

- Early prospective randomized controlled trials under- taken at our center suggested maximal benefit from assisted hatching in individuals over the age of 38 years, and specifically for patients with thickened zonae
- Stein et al. reported that partial zona dissection resulted in a significant improvement in implantation and pregnancy rates in women older than 38 years of age who had a history of RIF

- Clinical pregnancies per cycle were significantly elevated in patients undergoing assisted hatching where either age (31% vs. 10%) or repeated failure (36% vs. 17%) was the indication

- Hsieh et al. reported that hatching with a diode laser provided greater benefit than chemical- assisted hatching in older patients
- Given the heterogeneity of techniques and the wide range of published evidence, no specific assisted hatching technique has been established as the gold standard for patients with RIF.

- Endometrial scratch as a method for fostering implantation.
- The method is "healing process" that allows for release of cytokines and other growth factors that facilitate implantation.
- Mechanical endometrial injury prior to controlled ovarian hyper- stimulation has been proposed as a method to induce decidualization and attract cytokines, growth factors, LIF, and other immune modulators to the endometrium

- Endometrial biopsy on days 8, 12, 21, and 26 of the cycle preceding IVF, with the data suggesting a significant improvement in subsequent implantation rates (27.7% vs. 14.3%) following repeated biopsies.

Co-culture

- Co-culture of *in vitro*-derived embryos with either tubal epithelium, endometrial epithelium, granulosa, or cumulus cells has been proposed to foster more supportive culture conditions
- Variable reported success rates with these techniques are likely attributable, at least in part, to differences in cell lines, maintenance of cells, and various environmental factors within each laboratory.

- Vero cells (from monkey kidney epithelium) and bovine oviductal epithelium have both been noted to improve embryo quality and pregnancy rates in poor-prognosis patients
- Co-culture of human embryos with buffalo rat liver cells also suggested a favorable trend (34% vs. 28%) towards improved pregnancy rates in patients with prior failures
- Xeno-culture, however, poses both theoretical and practical infectious risks that make the use of various animal cells less than ideal for human embryos.
- Because of these potential risks, investigators have focused on utilizing either homologous or autologous human cells in co-culture systems.
- However, the risk of trans- mission of infectious agents along with Creutzfeldtakob disease limit the desirability of homologous techniques.

- Patients undergo an endometrial biopsy in the mid-luteal phase of a cycle preceding their IVF treatment cycle, and endometrial glandular epithelial and stromal cells are separated by differential sedimentation and plated until a monolayer is achieved.
- The cells are then frozen and later thawed during the patient's treatment cycle.
- An equal mixture of glandular epithelial and stromal cells is seeded into a four-well tissue plate containing Ham's F-10 medium supplemented with 15% patient serum.
- Embryos are introduced into the co-culture system after fertilization and maintained with the autologous endometrial cells until the day of transfer.

- Human endometrial co-culture has been beneficial to blastocyst development, presumably owing to a chemical cross-talk and paracrine signaling between embryo and endometrium
- The use of autologous endometrial cells for co-culture in patients with RIF was first reported by
- Simon et al. achieved a 39.2% blastocyst formation rate, an 11.8% implantation rate, and a 20.2% pregnancy rate with an autologous endometrial co- culture system in 168 cycles among patients with three or more failed implantation cycles
- Eyheremendy et al. similarly demonstrated benefit utilizing autologous endometrial cell coculture with day-3 transfer in patients with RIF

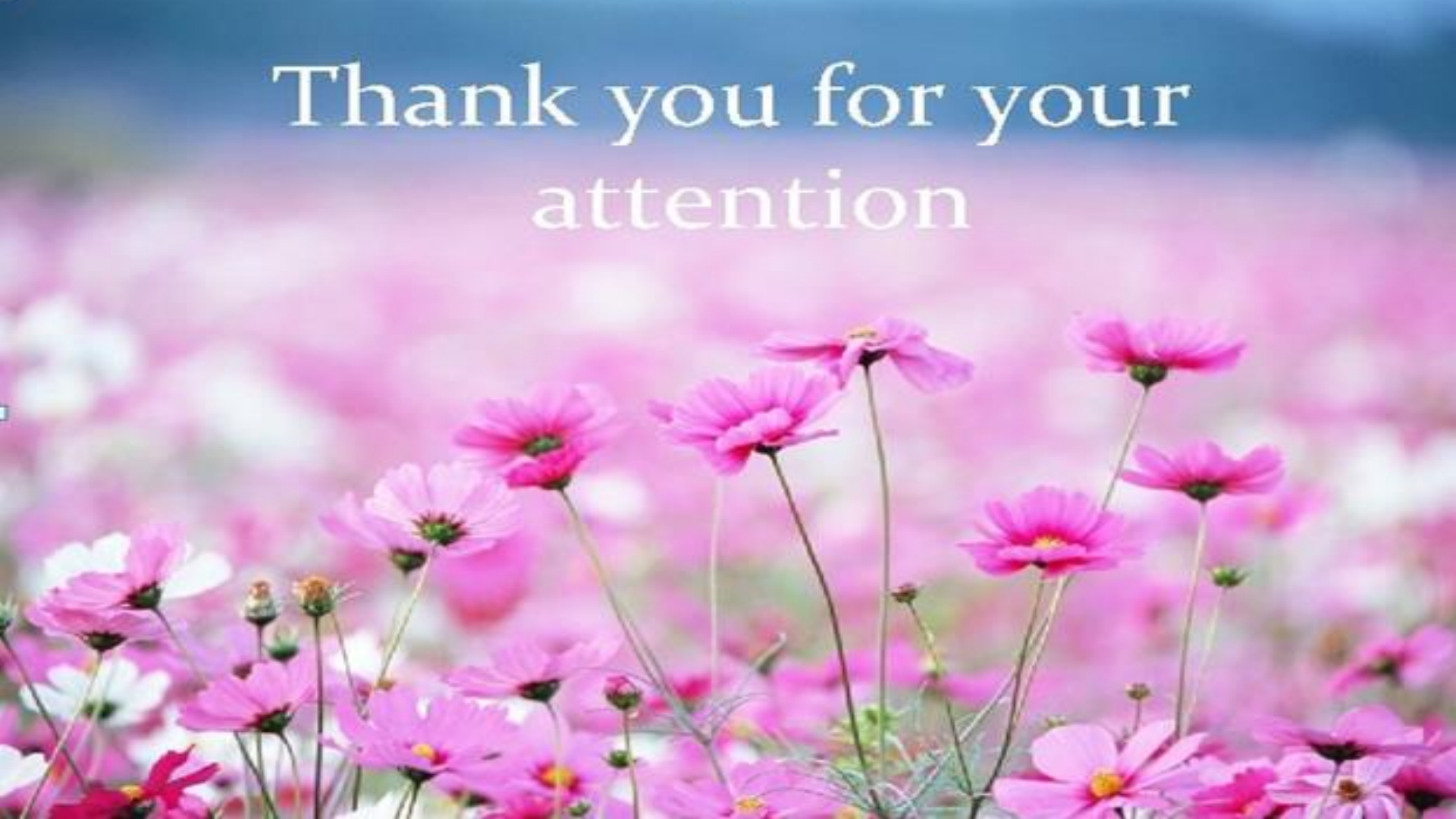
- In our own experience, sibling oocytes from RIF patients undergoing endometrial co-culture exhibit lower fragmentation and more blastomeres at the time of transfer as compared to traditionally cultured embryos
- Moreover, as potentially better embryo incubation techniques such as time-lapse microscopy at low oxygen tensions emerge, the incremental benefit of endometrial co culture remains to be further defined for these difficult RIF patients.

CONCLUSION

- Although treatment of patients with a history of RIF can be discouraging, techniques and methodologies striving to optimize IVF success in these patients continue to evolve.
- We must continue to investigate and elucidate factors that may prevent our patients from achieving live births.

- Further evaluation of embryo–endometrial and ideal timing of transfer into a receptive endometrium may lead to new treatments for patients experiencing RIF.
- Improved embryo culture and embryo analytic techniques may offer finer discernment of embryos with the greatest implantation potential.
- The physician caring for a patient or couple with RIF must carefully review the prior diagnostic workup, complete the investigation with appropriate analytic techniques, and offer empathy and encouragement while providing accurate counsel on the likelihood of success.

- Despite optimisation of treatment protocols and advances in laboratory, technologies, the management of imprecisely defined RIF poses a major clinical challenge.
- We propose a simple algorithm to calculate theoretical cumulative pregnancy rate.
- A more standardised and personalised definition of RIF will be helpful for patient counselling, clinical decision making and future research in this area.


Thank you for your
attention