REPEATED IMPLANTATION FAILURE

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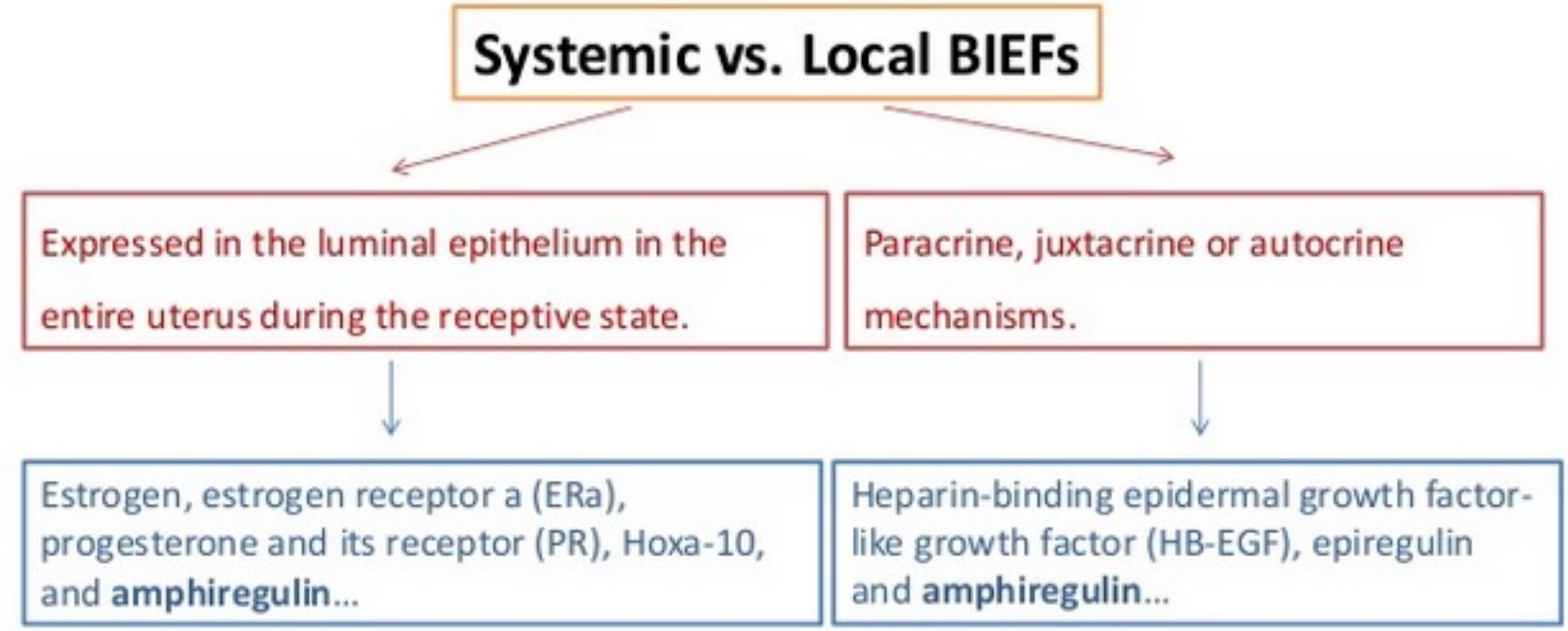
INTRODUCTION

Implantation is a unique complex dialogue between *receptive endometrium* and *healthy good quality embryo* where molecular and genetic evidence indicates that *ovarian hormones* together with *locally produced signaling molecules*, including cytokines, growth factors, homeobox transcription factors, lipid mediators and morphogen genes, function through *autocrine*, *paracrine* and *juxtacrine* interactions .

(Dey et al., 2004).

Blastocyst **I**mplantation **E**ssential **F**actors (BIEFs)

The blastocyst implantation essential factors **(BIEFs)** are a *collective name* given to the molecules that are indispensable for successful implantation of a **mature** and **healthy** **blastocyst**.



Koji Yoshinaga 2010

- In assisted conception treatment, implantation is considered to be successful when an embryo has produced an intrauterine gestational sac, detectable by ultrasound, usually about 3 weeks after oocyte retrieval or about 5 weeks of gestation.
- Implantation failure refers to the failure of the embryo to reach a stage when an intrauterine gestational sac is recognized by ultrasound.

(Coughlan et al. 2014)

Despite significant development in ICSI biotechnology, up to 70% of embryo loss occurs at time of Implantation

(Deke IN et al 2010).

DEFINITION

- No universally accepted definition for RIF.
- **Failure to achieve a clinical pregnancy after transfer of at least 4 good-quality embryos in a minimum of 3 fresh or frozen cycles in a woman under the age of 40 years** (Coughlan et al. 2014)
- The term 'recurrent implantation failure' is a subgroup of recurrent IVF failure and should not be used to replace the latter. (Ferraretti et al., 2011)

CAUSES OF RIF

A- GAMETE/EMBRYO FACTORS:

1. Parental chromosomal anomalies
2. Poor-quality oocyte
3. Poor-quality sperms
4. Zona hardening
5. Suboptimal culture conditions
6. Suboptimal embryo quality
7. Suboptimal ET

CAUSES OF RIF

B- ENDOMETRIAL FACTORS

1. Anatomic causes: Polyp, fibroid, adenomyosis, intrauterine adhesions, uterine septum
2. Impaired function: Thin endometrium, Altered expression of adhesive molecules
3. Thrombophilia e.g. APS ; still controversial
4. Immunological factors: decidualization of endometrial stromal cells & regulation of trophoblast invsion.

CAUSES OF RIF

C- OTHER CAUSES

1- Endmetriosis

2- Hydrosalpinges

3- Improper ovarian stimulation

CAUSES OF RIF (summary)

1- Gamete/embryo factors:

- oocyte quality - sperm quality
- parental chromosomal anomalies

2-Uterine factors: congenital or acquired

3- Hydro-salpinges

4- Immunological factors

5- Thrombo-philias

INVESTIGATIONS

Gamete and embryo factors

1- Ovarian function tests ; ovarian reserve tests such as basal FSH, AMH and AFC counts .

2- Sperm DNA integrity testing; sperm DNA fragmentation index (DFI). Males with DFI >/= 30% are usually associated with RIF

3- Kryotyping: Although only a small proportion of couples with RIF have abnormal karyotype results , the rate is higher than that of the general population, suggesting an association between the two conditions.

investigations

Uterine factors

In women with RIF, thorough investigations must be carried out to exclude any uterine pathology contributing to the clinical problem.

1- ultrasound 2- hysterosalpingography

3- sonohysterography 4- hysteroscopy

5- combined lapaoscopy & hysteroscopy

investigations

Table 1 Investigation of recurrent implantation failure.

Recommended investigations	*Investigations of research value*
Hysteroscopy	Hereditable/acquired thrombophilia
Hysterosalpingography	Sperm DNA fragmentation
Pelvic ultrasonography	
Parental karyotype	
Ovarian reserve and function: FSH, anti-Müllerian hormone, antral follicle count	

MANAGEMENT

A multidisciplinary approach should be adopted in the management of a couple with RIF. It should involve *not only* an experienced fertility specialist but also a senior embryologist and, where appropriate, a reproductive surgeon or a counselor.

MANAGEMENT

A careful review of recent investigations including age of the woman, AFC, basal FSH measurement, AMH concentration, number of follicles produced in response to stimulation, number of oocytes retrieved, the proportion of immature oocytes, fertilization rate, the proportion of good-quality embryos and the total number of good-quality embryos transferred should be noted.

MANAGEMENT

LIFESTYLE CHANGES

- lifestyle changes which could improve the likelihood of treatment success.

1. Smoking; stop
2. Body mass index : should be [between ≥ 19kg/m^2 and ≤ 29kg/m^2]
3. Alcohol consumption should be decreased or stopped.

MANAGEMENT

Ovarian stimulation protocol

-Revise previous protocols, if satisfactory ok, if not modify regarding the gonadotropin dose or protocol .

- Women with endometriosis or adenomyosis may respond better to ultra-long protocol using the agonist for few months before gonadotropin.

MANAGEMENT

SPERM DNA FRAGMENTATION

1. oral antioxidant treatment
2. select spermatozoa with low levels of DNA damage from the ejaculated semen samples using annexin-V columns technique or confocal light absorption scattering spectroscopy (CLASS) technology
3. Use of testicular spermatozoa (TESA) instead of ejaculated spermatozoa for ICSI

MANAGEMENT

Improving embryo quality and selection

Even though RIF refers to those who fail to achieve a clinical pregnancy despite the transfer of good-quality embryos, embryo factors still play a part because the currently used methods of embryo selection are *not always reliable*.

MANAGEMENT

Improving embryo quality and selection

Time – Lapse System

- Continuous (**Dynamic**) imaging monitoring , both morphological & developmental kinetics of the cultured embryos rather than snap shot (**Static**) assessment which have limited predictive value , ESHRE/ALPHA 2011.

- Processing of the data at the day of transfer with **Cell tracking software algorithm** or digitally displayed on a monitor.

- Selection through development of **Multivariable Model** depending on morphokinetic parameters which classify embryos according to their probability of implantation .

MANAGEMENT

Improving embryo quality and selection

Time – Lapse System

- **Advantages:**
  - **Maintains stable environment.**
  - **Improves embryo selection:**
    - ❑ Timing of cell division.
    - ❑ Interval between cell cycle.
    - ❑ Dynamic pronuclear pattern .
    - ❑ Presence of multinucleation & fragmentations .
    - ❑ Blastomeres symmetry.
- **Disadvantages:**
  - **Expensive with exposure of embryo to UV light.**

MANAGEMENT

Improving embryo quality and selection

Time – Lapse System

Clinical outcomes following selection of human pre-implantation embryos with time-lapse monitoring: a systematic review concluded that;

insufficient high-quality evidence to support the use of TLM in routine clinical practice. selection of embryos by TLM should remain an experimental strategy subject to institutional review and approval.

A common nomenclature should be adopted in all future TLM studies.

Daniel J. Kaser and Catherine Racowsky

Human Reproduction Update, Vol.20, No.5 pp. 617 –631, 2014

MANAGEMENT

Blastocyst transfer

Several studies have suggested that extending embryo culture to day 5 or 6 in order to transfer the embryo at the blastocyst stage increases the implantation rate, however it does not increase CPR or LBR.

MANAGEMENT

Assisted Hatching

- Assisted hatching involves the artificial thinning or breaching of the zona pellucida and has been proposed as one technique to improve implantation and pregnancy rates following IVF
- Increased risk of monozygotic twins.

MANAGEMENT

Pre-implantation genetic diagnosis (PGD/PGS/PGT)

- The value of PGD in RIF is controversial.
- There is no evidence to suggest that the embryos produced by women with RIF are more likely to be abnormal.
- The frequency of aneuploidy in embryos from women with RIF is (67%) **(Pehlivan et al., 2003)** while it was (64%) in women without the condition **(Baart et al., 2006).**

MANAGEMENT

Pre-implantation genetic diagnosis (PGD)

Recent studies suggested that detailed characterization of blastocyst cytogenetics using methods such as comparative genomic hybridization (CGH) and single-nucleotide polymorphism microarrays and next generation sequence (NGS) with a view to detecting and preferentially transferring euploid normal embryos had improved implantation rates in couples with history of RIF **(Fragouli et al., 2011)**

MANAGEMENT

Embryo transfer (ET)

previous embryo transfers should be reviewed, paying particular attention to any technical difficulties encountered such as a procedure taking longer than usual, causing significant pain or requiring change of catheter, cervical dilatation or use of a tenaculum.

MANAGEMENT
Embryo transfer (ET)

- **Ultrasound guidance**: ET should be performed under ultrasound guidance.
- **Trial ET/mock transfer**; should be done in cases in whom difficulty is anticipated. It is done on day of egg retrieval.
- **Transfer tips**: full bladder with AVF, empty bladder with RVF. The use of a rigid catheter may help to negotiate the cervix if difficulty is encountered with the use of a soft catheter.
- **Transfer method:** Alternative methods to trans-cervical embryo transfer include trans-myometrial and tubal transfer but should be reserved for cases which are extremely difficult or impossible.

MANAGEMENT
Embryo transfer (ET)

- **Irrigation & aspiration of cervical mucus**: The removal of cervical mucus is thought to improve pregnancy rates by preventing or minimizing bacteriologic contamination of the endometrial cavity and preventing cervical mucus occluding the catheter tip but it remains to be determined as to whether this practice improves pregnancy rates.
- **Sequential (double) embryo transfer :** The concept behind this strategy is to overcome the problem of **embryo–endometrium asynchronicity** as a potential cause of implantation failure. However there is no sufficient evidence to support this practice.
- **Transfer into the fallopian tube (ZIFT):** was suggested as management of cases of RIF, however, the high cost, requiring laparoscopy, anesthesia, plus advances in the culture media limits its value.

MANAGEMENT

The uterus

- Hysteroscopy;

- there is convincing evidence that hysteroscopy improves the outcome of women with RIF.
- Removal of endometrial polyps, submucous myomas, uterine septum, or intrauterine adhesions.

MANAGEMENT

The uterus

- Myometrial pathology:

- Intramural myoma: no consensus about its removal especially if < 4cm and not distorting the cavity.

- Adenomyosis; The role played by adenomyosis in reproductive failure is receiving increasing attention and is now recognized to be a cause of RIF. Unlike fibroid it is not amenable for surgical removal. The best management is ultra-long pituitary down-regulation.

MANAGEMENT

The uterus

Thin endometrium; (<7mm at time of HCG).

1. *Modified long protocol with exogenous estrogen therapy:*

6-8mg estradiole valerate or estradiole transdermal patch 400 µgm/day started on second day of menses of down-regulated cycle (after a week of GnRH agonist). ET is monitored with serial ultrasonography after 7days of estrogen therapy and thereafter every 3 or 4 days until the endometrium has grown to more than 5 mm. At this stage gonadotrophin may be started for ovarian stimulation.

(The Royal Hallamshire Hospital protocol)

MANAGEMENT

The uterus

Thin endometrium; (<7mm at time of HCG).

2. *Sildenafil* :

- Sildenafil citrate has also been proposed in the treatment of women with RIF associated with a thin endometrium based on improvement of blood supply.
- There is no sufficient evidence supporting its role in improving implantation in couples with RIF.

MANAGEMENT

The uterus

Thin endometrium; (<7mm at time of HCG).

3. Luteal support with GnRHa :

Quoblan et al 2008 suggested that women who received GnRHa on day of oocyte retrieval, on the day of embryo transfer and 3 days later appeared to have significantly higher estradiol and progesterone concentrations, thicker endometrium and higher implantation and pregnancy rates than those who received placebo.

MANAGEMENT

The uterus

Thin endometrium; (<7mm at time of HCG).

4. Endometrial perfusion with granulocyte colony-stimulating factor:

5. Intrauterine autologous peripheral blood mononuclear cells (PBMC)

MANAGEMENT

Removal of hydrosalpinges

Salpingectomy:

-There is now good evidence that the removal of hydrosalpinges improves the implantation and live birth rates in women undergoing IVF.

-when carrying out salpingectomy, to diathermize and incise as close to the Fallopian tube as possible and as far away from the ovary as possible to avoid disruption to the ovarian blood supply.

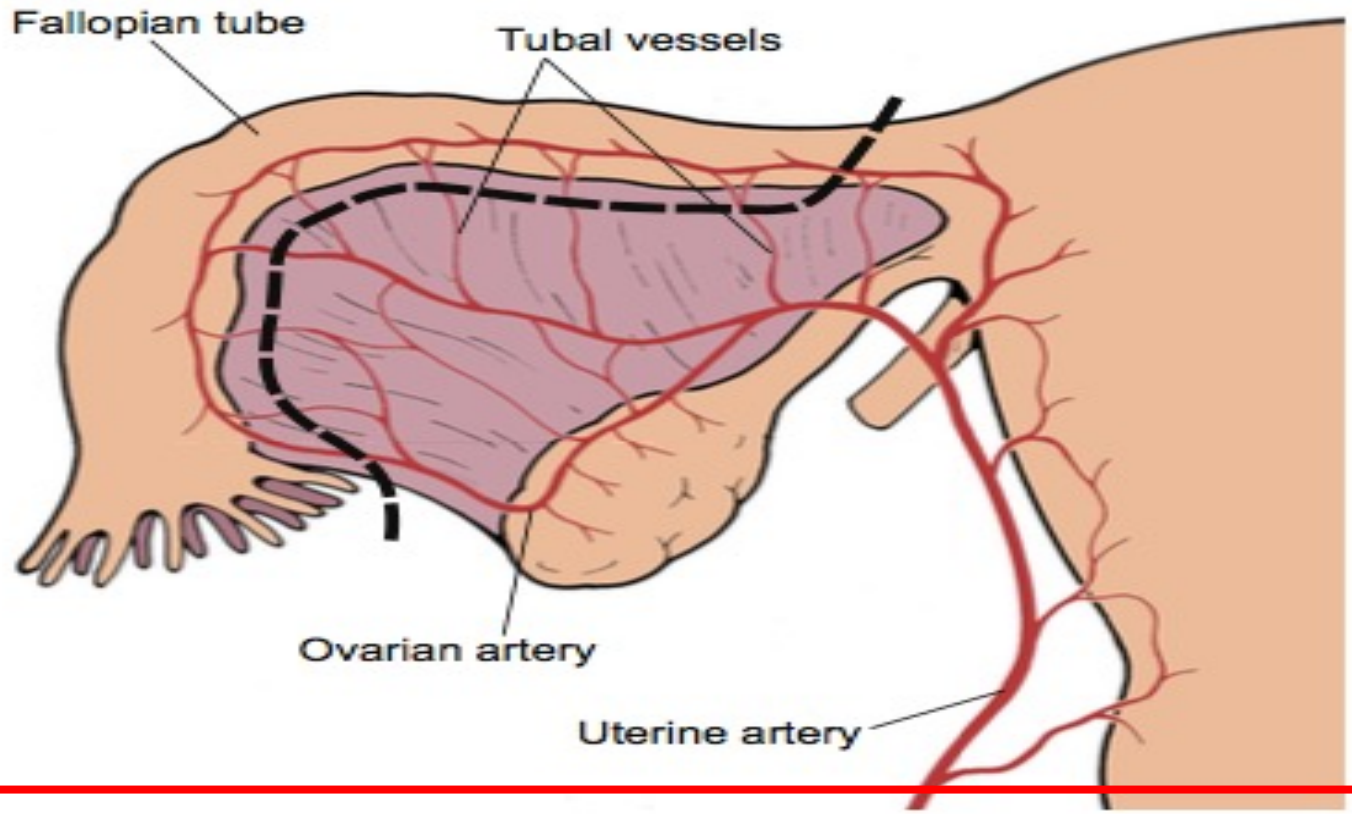


Figure 2 Salpingectomy for hydrosalpinx to improve the implantation rate. Diathermy and incision should be made as close as possible to the under surface of the tube and as far as possible from the ovary to avoid compromising the ovarian supply, which may in turn reduce ovarian response to stimulation during IVF treatment.

MANAGEMENT

Removal of hydrosalpinges

Salpingostomy:

-Salpingostomy may be a possible alternative as it not only 'removes' the hydrosalpinges but also produces the possibility of natural conception. The intrauterine pregnancy rate following salpingostomy has been reported by a number of investigators to be over 30%espicially if the tubal damage is minimal. The drawback is that recurrence rate is high.

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MANAGEMENT

Removal of hydrosalpinges

Tubal disconnection using bipolar diathermy:

-this is the most common technique used nowadays for treatment of hydrosalpiges before IVF cycles because of its simplicity and minimal effect on the ovarian blood supply.

- Its main disadvantage is that the hostile intra-fallopian fluid is not eliminated.

. Transvaginal aspiration of hydrosalpinx is not recommended

MANAGEMENT
Endometrial scratching

Endometrial injury in women undergoing assisted reproductive techniques

Carolina O Nastri,Sarah F Lensen,Ahmed Gibreel,Nick Raine-Fenning,Rui A Ferriani,
Siladitya Bhattacharya,Wellington P Martins

First published: 22 March 2015

Moderate-quality evidence indicates that endometrial injury performed between day 7 of the previous cycle and day 7 of the embryo transfer (ET) cycle is associated with an improvement in live birth and clinical pregnancy rates in women with more than two previous embryo transfers.

Evidence from well-designed trials that avoid instrumentation of the uterus in the preceding three months, do not cause endometrial damage in the control group, stratify the results for women with and without recurrent implantation failure (RIF) and report live birth.

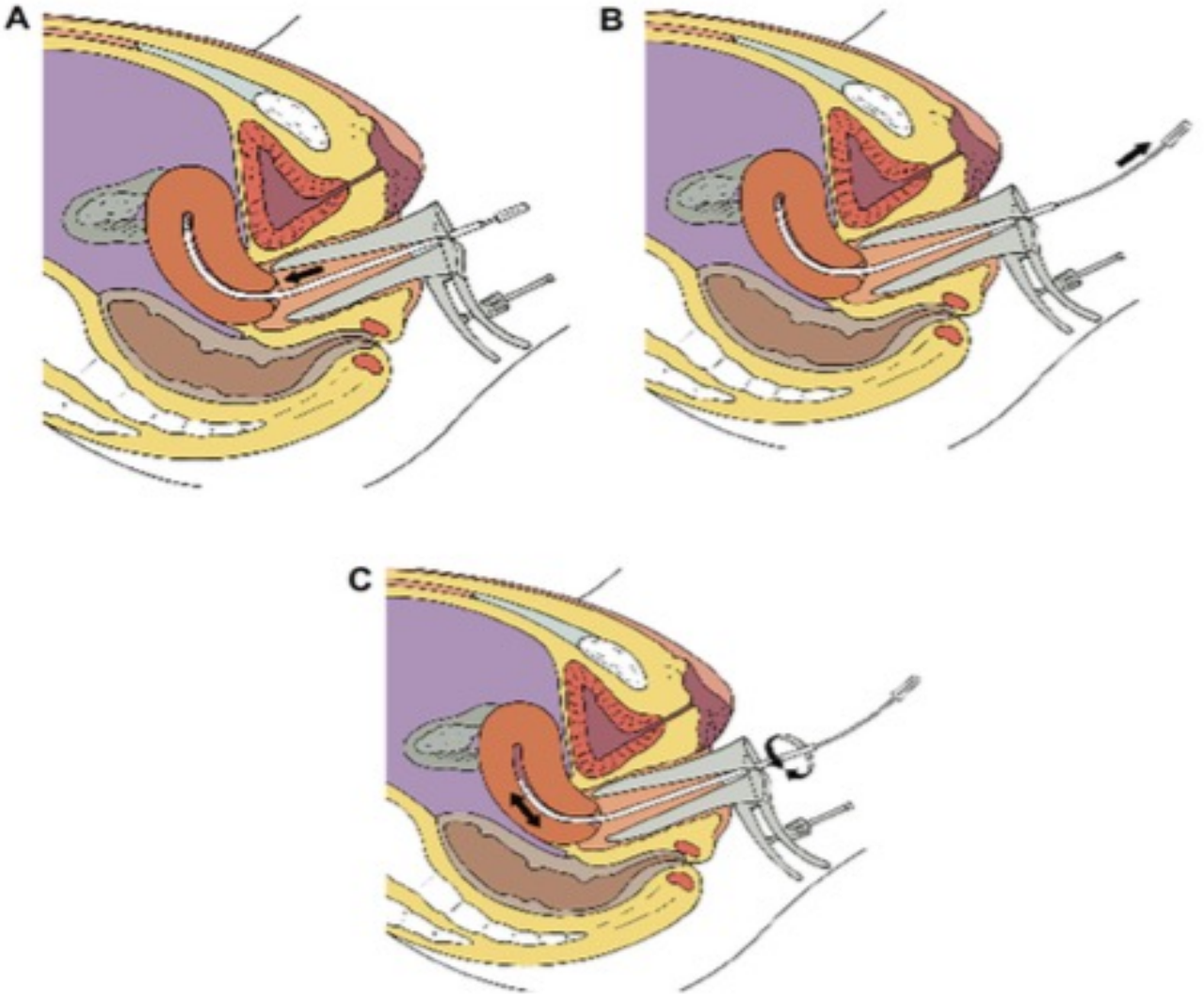


Figure 3 Endometrial scratch to improve implantation rate in women with recurrent implantation failure. (A) First, the pipelle sample is inserted until it reaches the fundus. (B) The inner plunger is withdrawn to apply a suction force to the endometrial cavity. (C) Endometrial scratch of the superficial layer of the endometrium is performed with the use of a 'hoovering' movement, combining a rotational and in-and-out movement of the pipelle sampler several times.

MANAGEMENT
Cellular Mediators & Cellular Treatments

Intravenous immunoglobulins (IVIG):

Before oocyte retrieval , 0.2 – 0.4 gm./kg, may be repeated.

Data show some improvement but overall this SR doesn't support its use with low quality evidence, (Bolanski et al 2014).

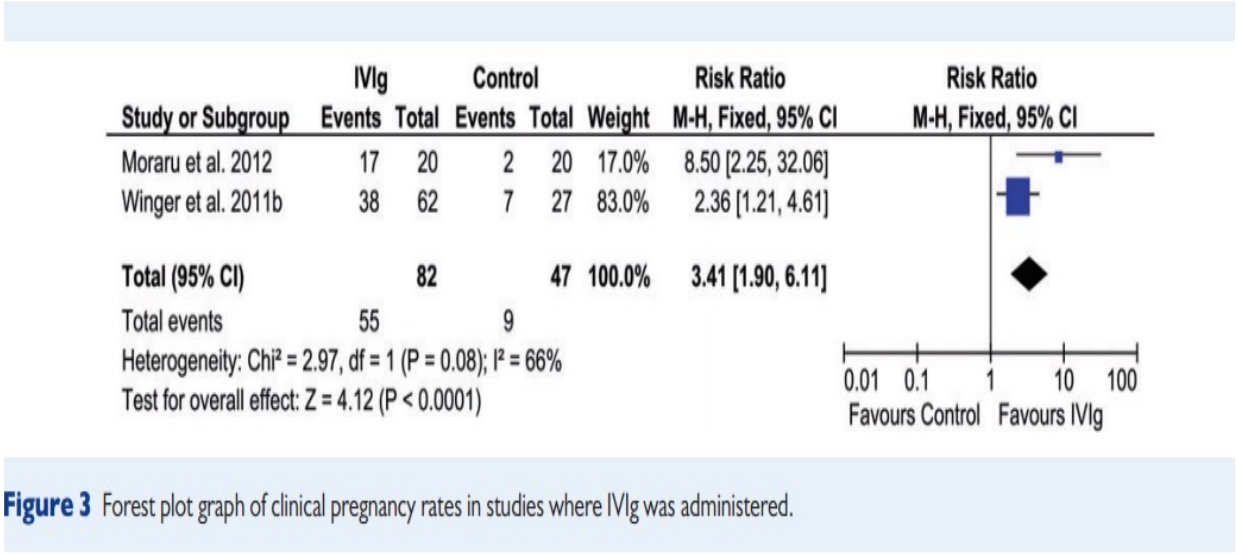


Figure 3 Forest plot graph of clinical pregnancy rates in studies where IVIg was administered.

MANAGEMENT
Cellular Mediators & Cellular Treatments

- **Leukeamia inhibitory factor:**

Before embryo transfer , SC 150 ug twice daily for 7 days,(Brinsden 2009).

- **Granulocyte colony –stimulating factor:**

On day of HCG or luteal phase ore both, local or systemic,(Gleicher 2013).

- **Tumor necrosis factor α antagonist**:

at time of implantation, (Benschop 2012).

MANAGEMENT
Other (empirical) Drugs

Heparin: Unfractionated or LMWH during COS or luteal phase

Effect of heparin on the outcome of IVF treatment: a systematic review and meta-analysis.

(Seshadri S1, Sunkara SK, Khalaf Y, El-Toukhy T, Hamoda H, Reprod Biomed Online. 2012 Dec;25(6))

- **Randomized trials showed ,No improvement in the clinical pregnancy rate or the live birth rate. Role of heparin in this context requires Further evaluation in adequately powered randomized studies.**

- **Heparin for assisted reproduction**

(Muhammad A Akhtar , 17 August 2013)

It is unclear whether peri-implantation heparin in assisted reproduction treatment (ART) cycles improves live birth and clinical pregnancy rates in subfertile women, Results do not justify the use of heparin in this context, except in well-conducted research trials.

NSAID:

Around time of implantation, **Not recommended for use due to lack of evidence** from the current data, Siristatidis 2012.

MANAGEMENT
Other (empirical) Drugs

- **Aspirin**

Some centers offer empirical use of aspirin or heparin in women with RIF. A recent systematic review and meta-analysis on the use of low-dose aspirin showed no benefit of its use in IVF programmes (Gelbaya et al., 2007).

A subsequent prospective, randomized, double-blind, placebo-controlled trial involving 201 couples concurred with the conclusion of the earlier meta-analysis (Dirckx et al., 2009). There is good evidence to suggest that aspirin should not be used in women with RIF.

MANAGEMENT
Other (empirical) Drugs

- **Fat emulsion (intra lipid):**

Around time of implantation, **controlled large –scale studies are needed before recommending its routine use**, (Shreeve 2012) .

- **Glucocorticoids:**

Around time of implantation, **No improvement in pregnancy outcome , individualized**, (Boomsma 2012) .

- **Vasodilators:**

No evidence supporting its routine use at or around the time of embryo transfer, (Nardo LG et al 2011).

No sufficient or low quality evidence about increase in CPR &LBR ,(Gutarra – Vitchez 2014) .

- **Intrauterine HCG flushing:** no sufficient evidence

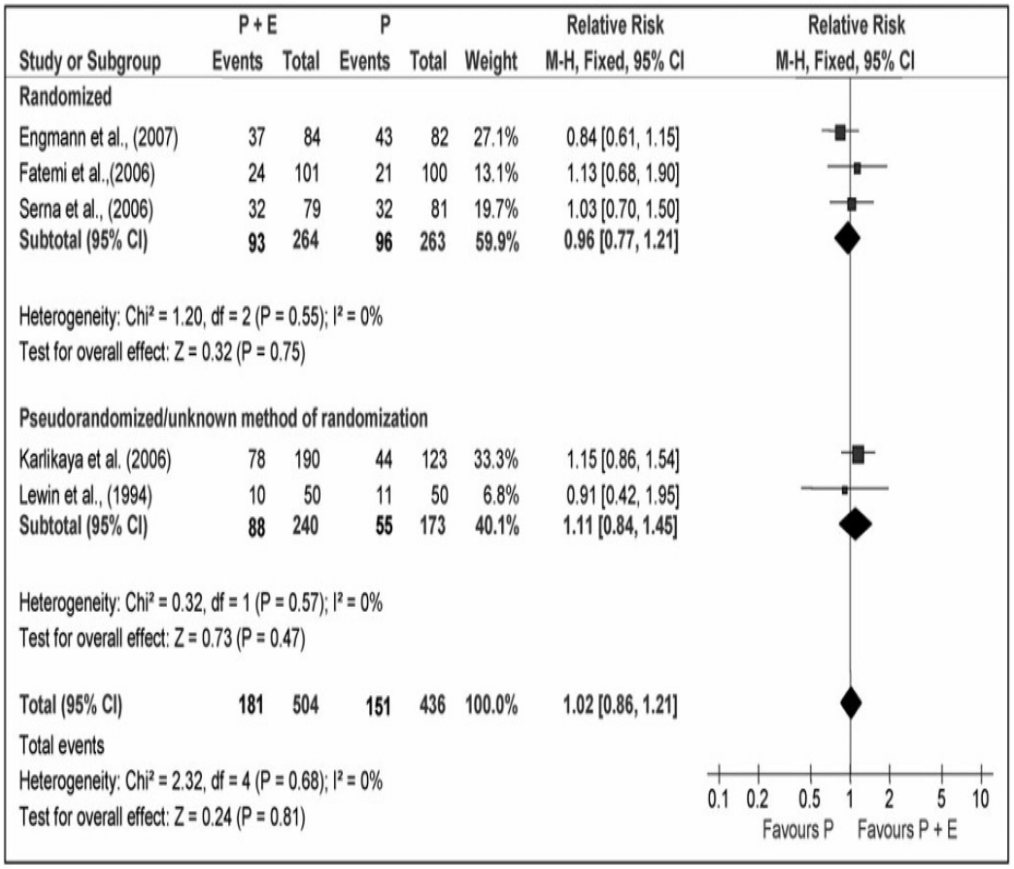
MANAGEMENT
Other (empirical) Drugs

- **Estrogen administration:**

During COS or luteal or both might **increase** endometrial blood flow and improving receptivity, (Siman 2012).

Human Reproduction Vol.23, No.6 pp. 1346–1354, 2008 doi:10.1093/humrep/den115
Advance Access publication on April 12, 2008

Estrogen addition to progesterone for luteal phase support in cycles stimulated with GnRH analogues and gonadotrophins for IVF: a systematic review and meta-analysis



British Fertility Society Policy and Practice Committee: Adjuvants in IVF: Evidence for good clinical practice. Hum Fertil (Camb). 2015

Nardo LG1, El-Toukhy T, Stewart J, Balen AH, Potdar N.

Evidence-based guidance and recommendations regarding the use of immunotherapy, vasodilators, uterine relaxants, aspirin, heparin and estrogen as adjuvants in IVF. Unfortunately despite the lapse of 5 years since the last publication, **There is still a lack of robust evidence for most of the adjuvants searched and large well-designed randomized controlled trials are still needed.**

Conclusion

- RIF should be defined as the failure to achieve a clinical pregnancy after transfer of at least 4 good-quality embryos in a minimum of 3 fresh or frozen cycles in a woman under the age of 40 years.
- Women with RIF should be offered appropriate investigations to rule out an underlying cause for the repeated failure .
- The main treatment strategy in couples with RIF is to *improve the quality of the embryos* transferred and the receptivity of the endometrium.

Conclusion

- The following recommendations should be considered in the management of couples with RIF. The levels of evidence available in the literature to support each recommendation are given in accordance with the guidelines published by the Royal College of Obstetricians and Gynaecologists (www.rcog.org.uk/guidelines)

Recommendations

- Hysteroscopy should be carried out to exclude any intra- cavity uterine pathology; it has been shown to improve out- come (evidence level 1+).
- Submucosal fibroids have been shown to reduce implantation, pregnancy and live birth rates; removal of submucosal fibroids improves implantation rate (evidence level 1+).

Recommendations

- Appropriate investigations should be carried out to exclude hydrosalpinx as it has been shown to reduce implantation rate, increase miscarriage rate and reduce live birth rate; removal of hydrosalpinges has been shown to improve the outcome (evidence level 1++).

Recommendations

- Endometrial polyps should be removed; although there is no data on its impact on women undergoing IVF, it has been shown to improve outcome in women undergoing intrauterine insemination (evidence level 1-□).
- Endometrial scratch should be considered in the luteal phase of the cycle immediately preceding IVF treatment; it improves implantation rate and outcome in women with unexplained RIF (evidence level 1-□).

Recommendations

- Uterine septum increases miscarriage rate; its removal improves outcome (evidence level 2+).
- The use of ultra-long protocol may improve outcome in women with endometriosis and adenomyosis (evidence level 3).

Recommendations

- Intramural fibroid of more than 5 cm should be removed (evidence level 3).
- Intrauterine adhesions are a recognized cause of thin endometrium not responding to ovarian steroid stimulation; if present, intrauterine adhesions should be removed (evidence level 4).

Recommendations

- A multidisciplinary approach should be adopted in the management of RIF (evidence level 4).
- Empirical therapies should, whenever possible, be considered only in the setting of carefully conducted clinical trials (evidence level 4).

Thank you