where **Science** meets **Compassion**

Advanced Fertility & Gynaecology Centre

www.advancefertility.in

Director **Dr. Kaberi Banerjee**

IVF & Recurrent Implantation Failure

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New Delhi

Definition

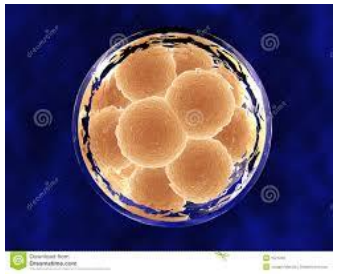
- Failure to achieve a clinical pregnancy after transfer of at least **4 good-quality embryos** in a minimum of **3 fresh or frozen cycles** in a woman **under the age of 40 years**

- Failure of implantation after transfer of at least **4 good-quality embryos or 2 blastocysts** after transfer in **2 cycles**.

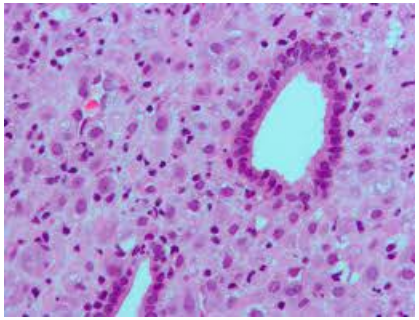
- *Failure could be caused by many different factors such as*
- ***inappropriate ovarian stimulation,***
- ***suboptimal laboratory culture conditions and***
- ***faults in embryo transfer techniques.***
- *These would usually result in a low pregnancy rate (PR) for the whole unit.*

Factors

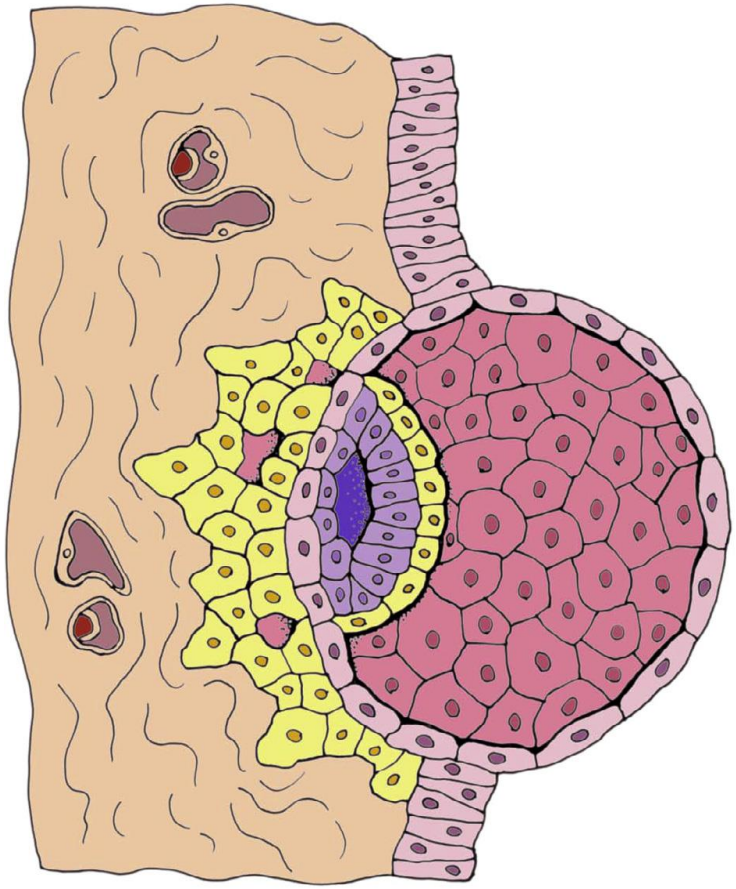
- Embryo factors



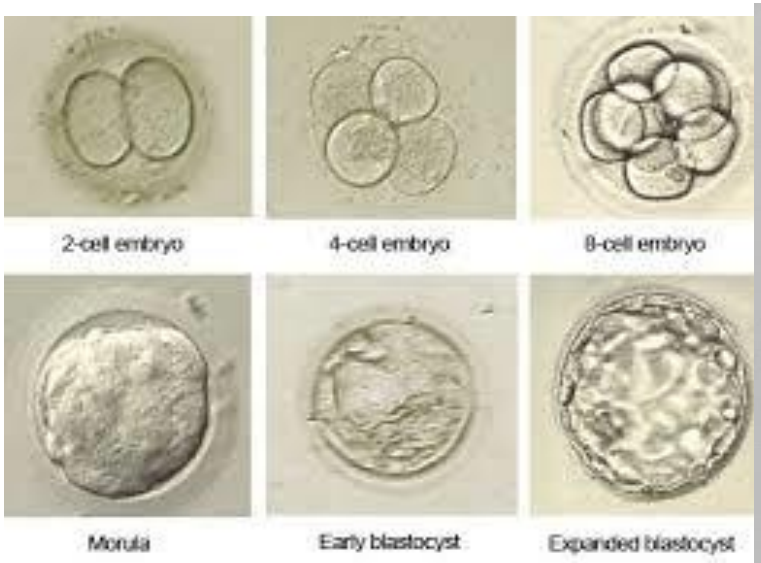
- Uterine factors

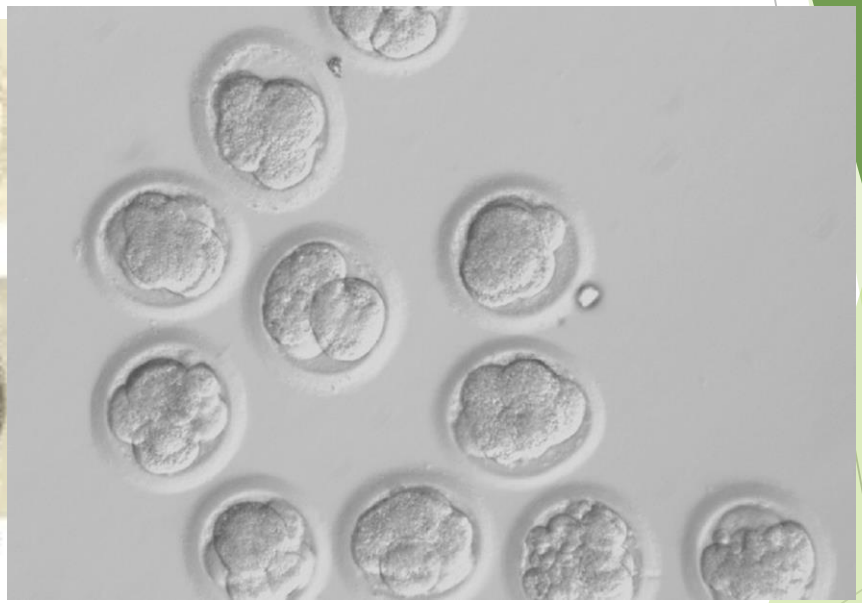


Oocyte+Sperm(Embryo)+Uterus= Implantation



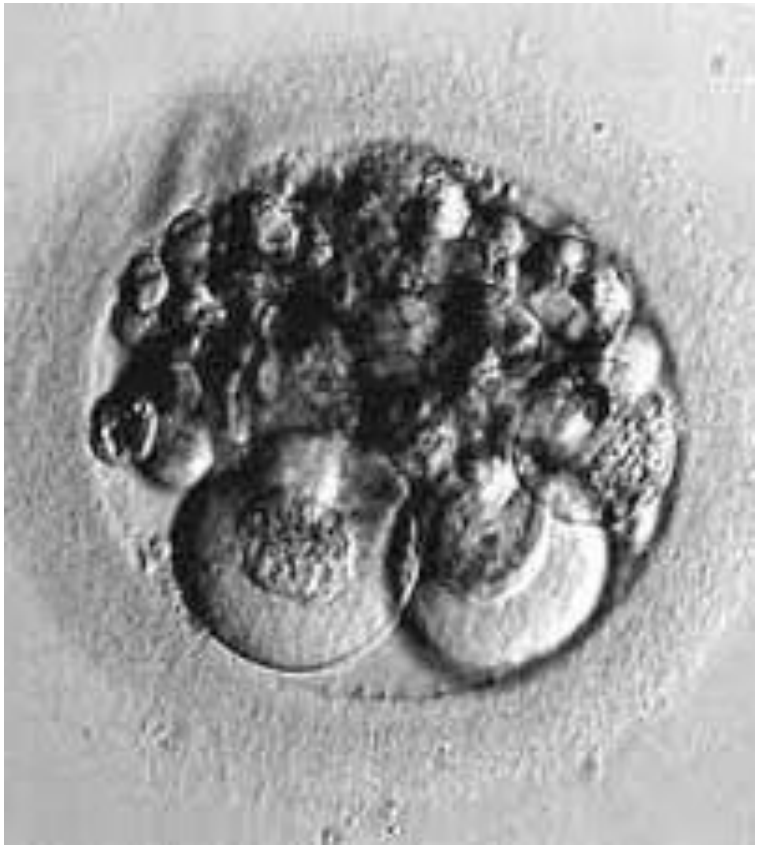
Cleavage-stage embryos





Day 5 assessment (Blastocyst stage)

Non-viable embryo



Oocyte Scoring

- Cumulus-oocyte complex scoring
  - Binary score (0 or 1)
  - ‘Good’ COC (score of 1) - expanded cumulus and a radiating corona
- Zona pellucida scoring
  - Colour or thickness of the zona pellucida.
- Perivitelline space - Presence of inclusions
- Polar body scoring
  - Presence or absence of the first polar body
  - Size of the polar body (Abnormally large polar body - risk of oocyte aneuploidy
- Cytoplasm scoring – Check for ‘granularity’ of cytoplasm
- Vacuolization - Large vacuoles (>14 microm in diameter) - fertilization failure.

Poor Oocyte Quality

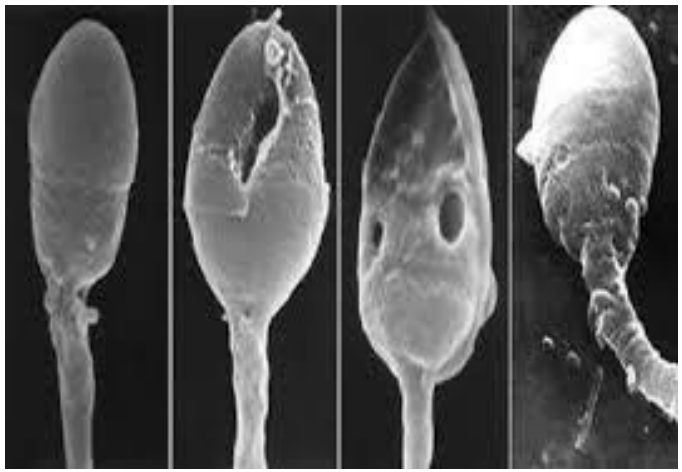
- Factors
- Higher age group
  - Increased chromosomal nondysjunction
  - Aneuploid embryos
  - Decrease in mitochondrial membrane potential and increase of mitochondrial DNA damage
- High FSH , low AMH and low AFC
- Aggressive ovarian stimulation protocols

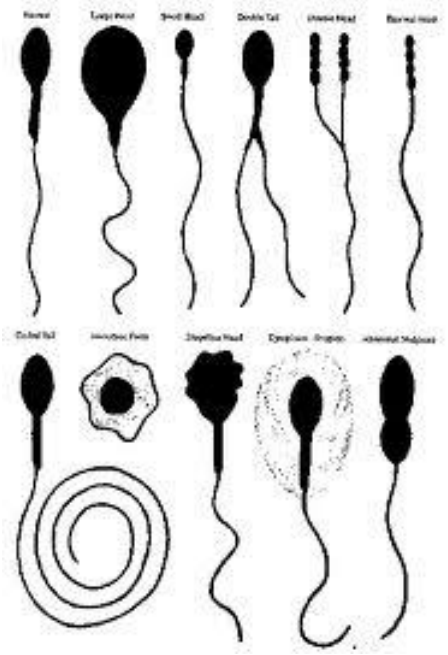
- Indicated by
  - Poor response to ovarian stimulation
  - Retrieval of few oocytes
  - High proportion of immature oocytes
  - Reduced fertilization rate
  - Low embryo utilization rate

Sperm

- Structure
- DNA Fragmentation

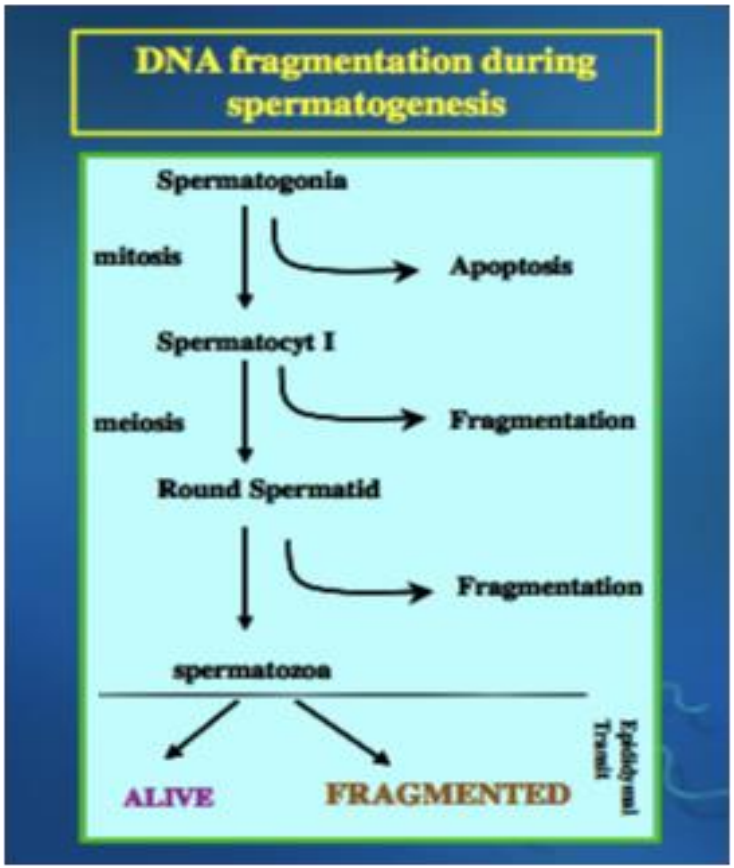
Sperm Structural Defects





- Infection
- Kartageners
- Globozoospermia

Sperm DNA fragmentation



Infection or inflammatory of genital tract

Varicocele

Testicular maldescent

Impaired spermatogenesis

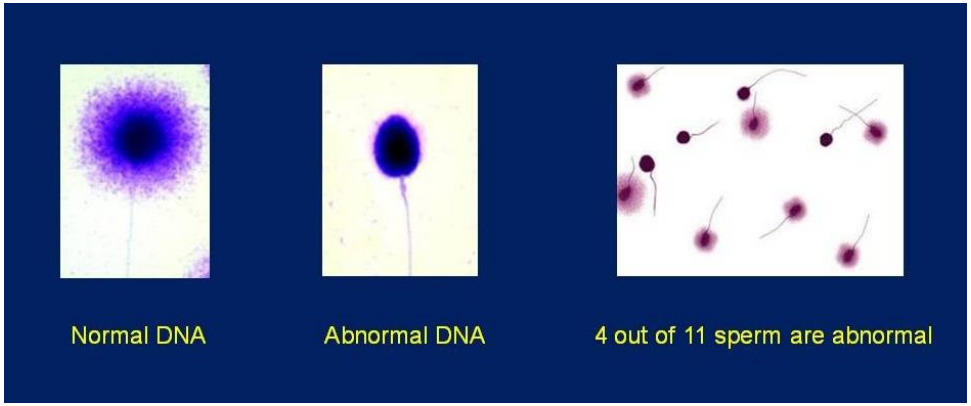
Radiotherapy or chemotherapy

Exposition to heat, chemical products

Idiopathic

Sperm DNA fragmentation

- Spermatozoa with fragmented DNA
  - ≤ 15 – good fertility potential
  - 15-25% - average
  - > 25% - poor fertility potential
- DFI > 25-30% - direct IVF/ICSI
- DFI> 30% - ICSI considered



Treatment

- Life style changes
  - Quit smoking
  - Stop alcohol consumption
  - Weight loss (In case of body mass index >29 kg/m2)

Treatment for Embryonic Factor (To Improve Egg quality)

- Age
- Good stimulation drugs and protocols
- DHEA
- Arginine

Treatment for Embryonic Factor (To Improve Egg quality)

- Ovarian stimulation protocol
- Proper gonadotropin dose selection according to ovarian reserve
- Addition of LH (> 35 years age)
- Ultra-long protocol for endometriosis and adenomyosis

EXPERT REVIEWS

Managing poor responders in IVF

Expert Rev. Obstet. Gynecol. 8(2), 121–134 (2013)

Key issues

- Poor responders have significantly lower clinical and live birth rates per age group in IVF.
- Increasing the gonadotropin dose is only effective up to 450 international units of follicle-stimulating hormone.
- Avoiding luteal gonadotropin-releasing hormone agonist or protocols using oral contraceptive pill suppression in favor of gonadotropin-releasing hormone flare and antagonist protocols have become standard for poor responders.
- Early transfer of day 2–3 embryos may be advantageous for poor responders.
- Precycle adjuvants, such as dehydroepiandrosterone, estrogen priming, clomiphene and letrozole flare protocols, may be beneficial but need further study.
- Oocyte donation remains the best option for poor responders with repeat IVF failure.

Hum Reprod Update. 2014 Jan-Feb;20(1):124-40. doi: 10.1093/humupd/dmt037. Epub 2013 Sep 29.

Individualization of controlled ovarian stimulation in IVF using ovarian reserve markers: from theory to practice.

La Marca A[1], Sunkara SK.

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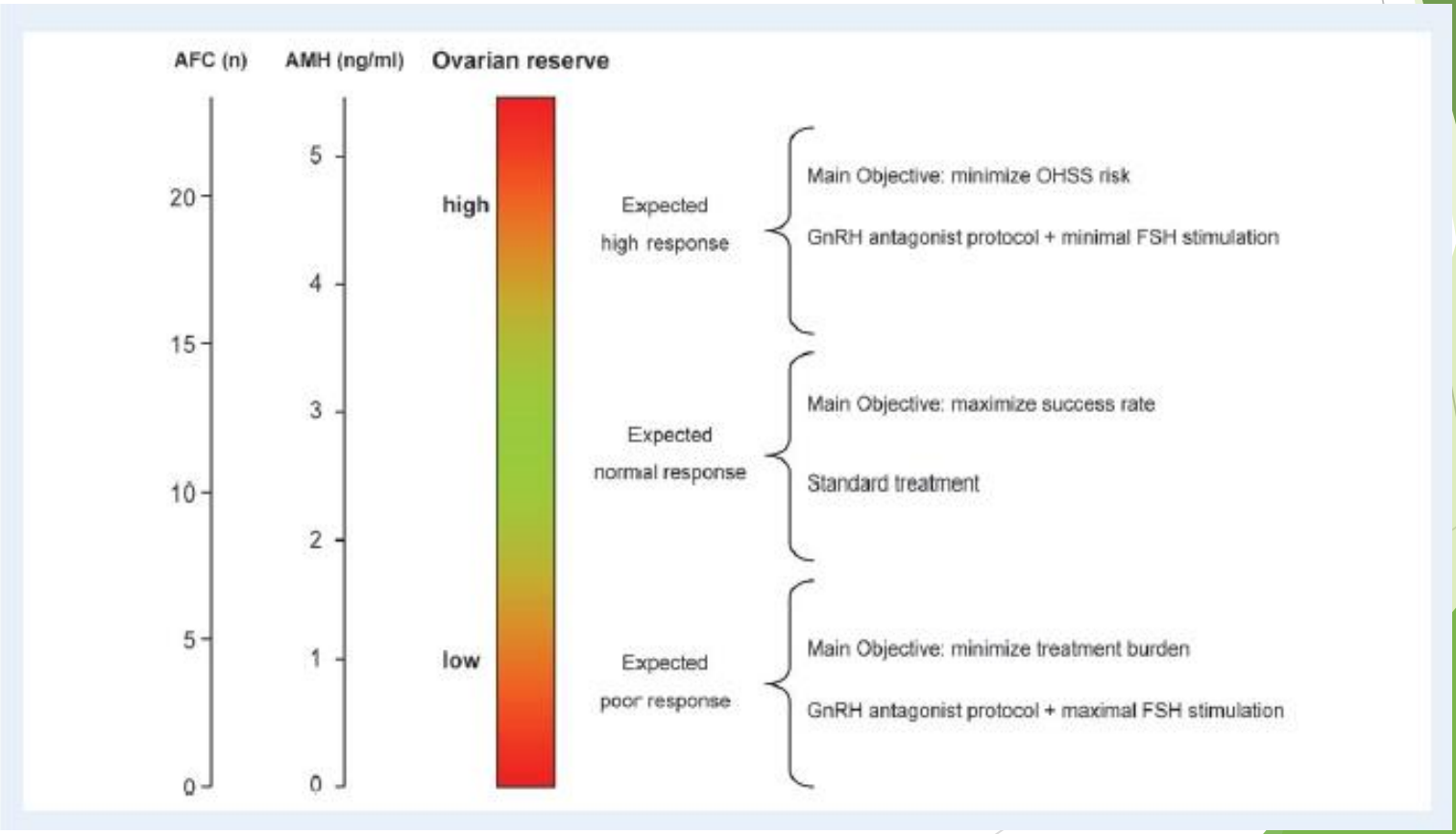
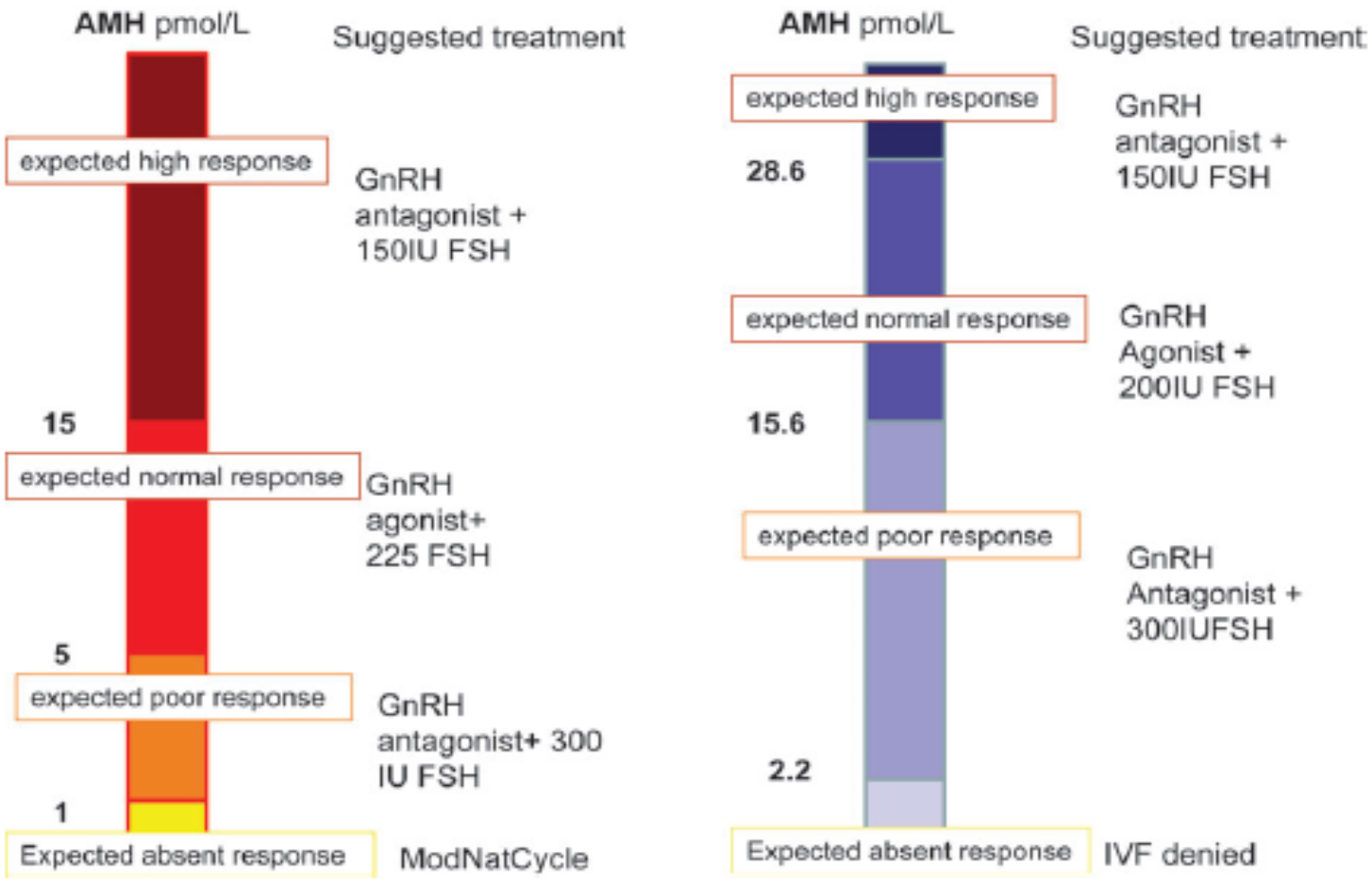
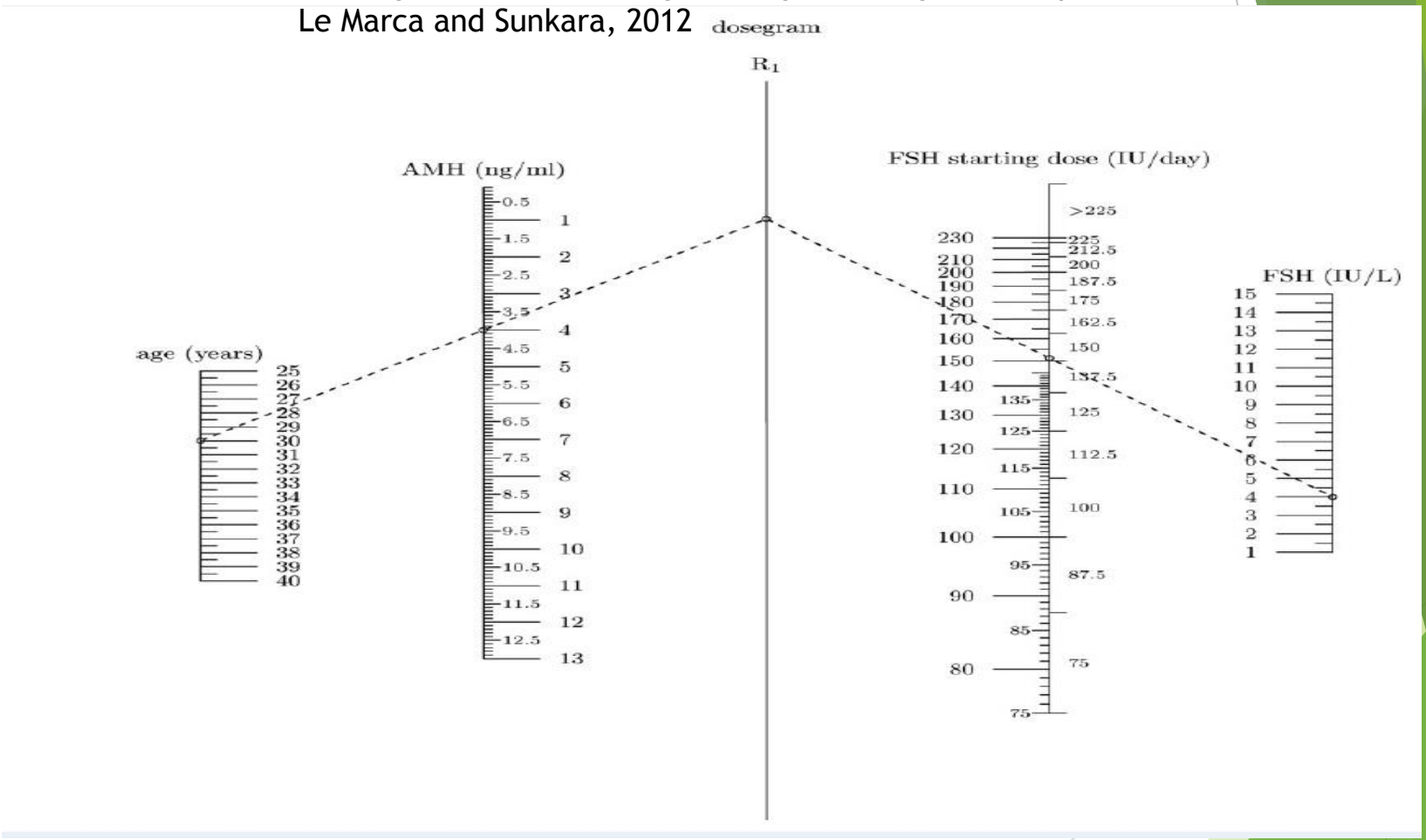




Strategic modelling of controlled ovarian hyperstimulation based on ovarian reserve markers

Normogram for calculating starting dose of gonadotropins in COS.
Le Marca and Sunkara, 2012

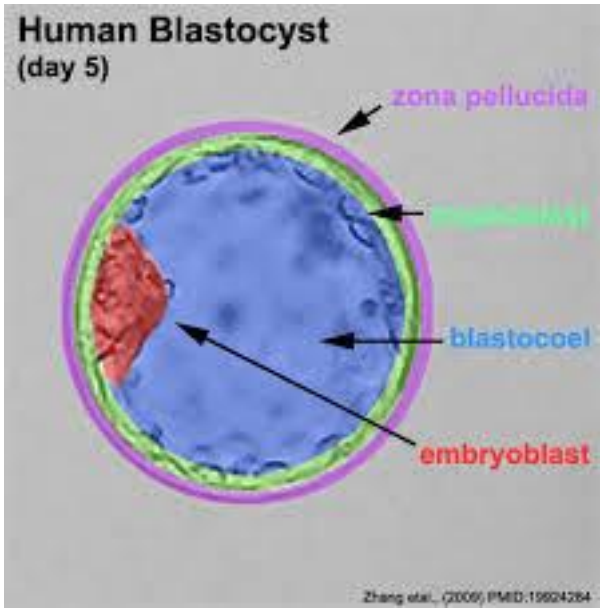


Treatment for Embryonic Factor (To Improve Sperm quality)

- Antibiotic treatment
- Anti oxidants
- Varicocele Surgery
- ICSI
- IMSI
- PGS

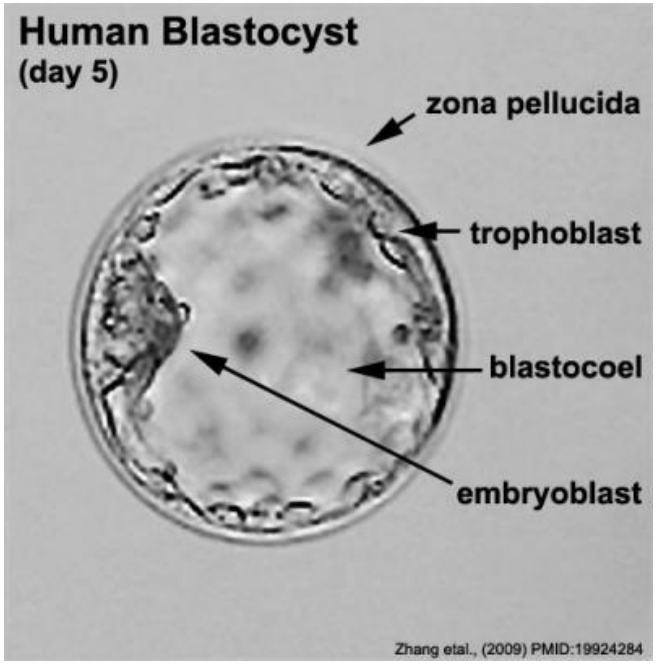
Treatment for Embryonic Factor

- Blastocyst transfer
- Assisted hatching
- Zygote intra-Fallopian transfer
- Metabolomics
- Use of Embryoscope
- Preimplantation genetic screening



Blastocyst Transfer

- Chromosomally competent embryos develop to blastocyst stage
- Physiological synchronization with uterine endometrium



Blastocyst culture and transfer in clinical-assisted reproduction: a committee opinion

The Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology

American Society for Reproductive Medicine, Birmingham, Alabama

CONCLUSIONS

- Evidence supports blastocyst transfer in "good prognosis" patients. Consideration is warranted to transfer of a single embryo given the high risk of multiples in this patient population.
- Blastocyst or cleavage-stage embryos can be used for unselected or poor prognosis patients as the pregnancy/live-birth rates are not significantly different; however, in these populations there is a higher risk of embryos not progressing to blastocyst stage resulting in fewer/no embryos available for transfer.

(Fertil Steril® 2013;99:667–72. ©2013

Assisted Hatching

- Artificial thinning or breaching of zona pellucida
- Laser, Mechanical, Chemical
- Indications: Thick Zona, Advanced Age, Frozen cycles.

Zygote intra-Fallopian transfer (ZIFT)

- Advantage
  - Zygotes comes in contact with numerous growth factors and cytokines in tubal fluid & attain to the uterus with greater synchronization
- Disadvantages
  - Need for GA, laparoscopy, theatre time and surgical equipment
  - Expensive procedure
  - Increased risk of ectopic gestation

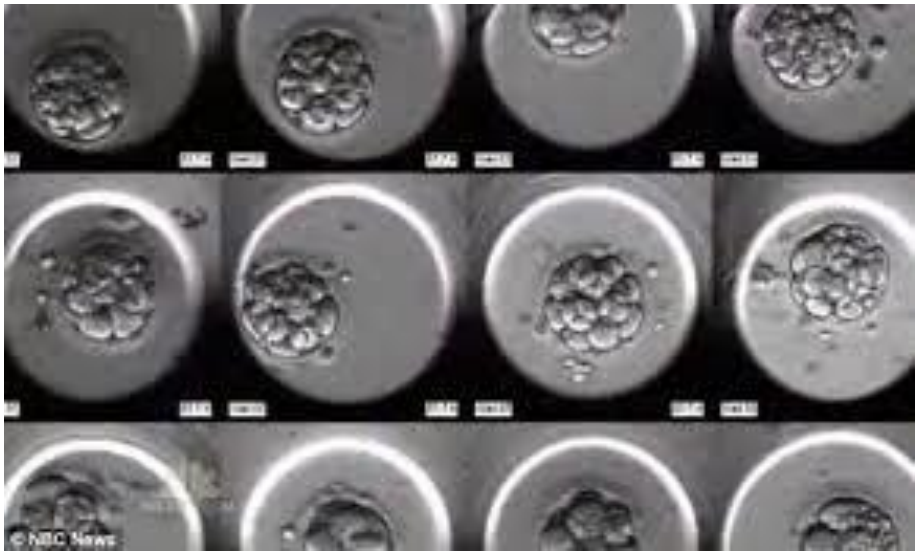
Embryo Metabolism

- Predictor of reproductive potential
  - Pyruvate and lactate metabolism
  - Glucose metabolism
  - Amino acid metabolism

Embryoscope - Time-lapse analysis

- Novel non-invasive method
- Dynamic morphometric assessment
- Monitor embryo development continuously
- Allows precise measurement of 1st cleavage time, PN fading
- Prevents from taking the dishes out of the incubator

Embryoscope



J Assist Reprod Genet (2015) 32:1025–1030
DOI 10.1007/s10815-015-0510-6

ASSISTED REPRODUCTION TECHNOLOGIES

A critical appraisal of time-lapse imaging for embryo selection: where are we and where do we need to go?

Catherine Racowsky[1] · Peter Kovacs[2] · Wellington P. Martins[3]

Conclusions The findings from this systematic review of the current evidence do not support routine use of time-lapse technology in clinical IVF. We therefore believe that the use of time-lapse imaging for embryo selection should remain experimental and that couples should not be subject to a surcharge for having their embryos cultured in a time-lapse imaging system. Future studies evaluating this technology in well-designed trials should be performed.

Pre-implantation genetic diagnosis (PGS)

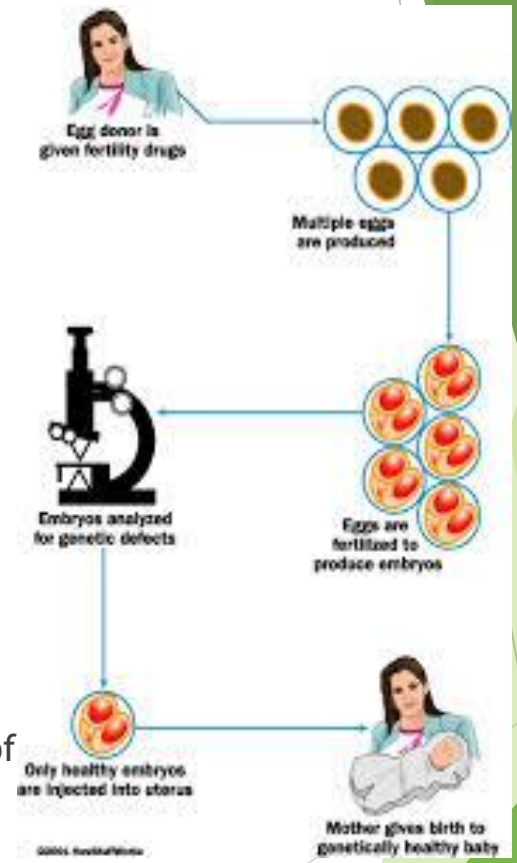
- Genetic analysis of a single cell from an embryo done in conjunction with in vitro fertilization (IVF)
- Genetic counselling before procedure
- To detect Chromosomal normalcy

PGS indications

- Recurrent miscarriages
- Maternal age older than 38
- Prior failure with IVF

PGS Method

- Ovulation Induction with drugs
- Egg Retrieval
- Fertilization
- Biopsy
  - Unfertilised and fertilised oocytes (for polar bodies, PBs)
  - On day three cleavage-stage embryos (for blastomeres – Preferable)
  - On blastocysts (for trophectoderm cells)
- Genetic Analysis (FISH, PCR,aCGH)
- Comparative genomic hybridization (CGH) can promote detection of genetic abnormalities (deletions or excess of DNA content) across the genome
- Embryo Transfer



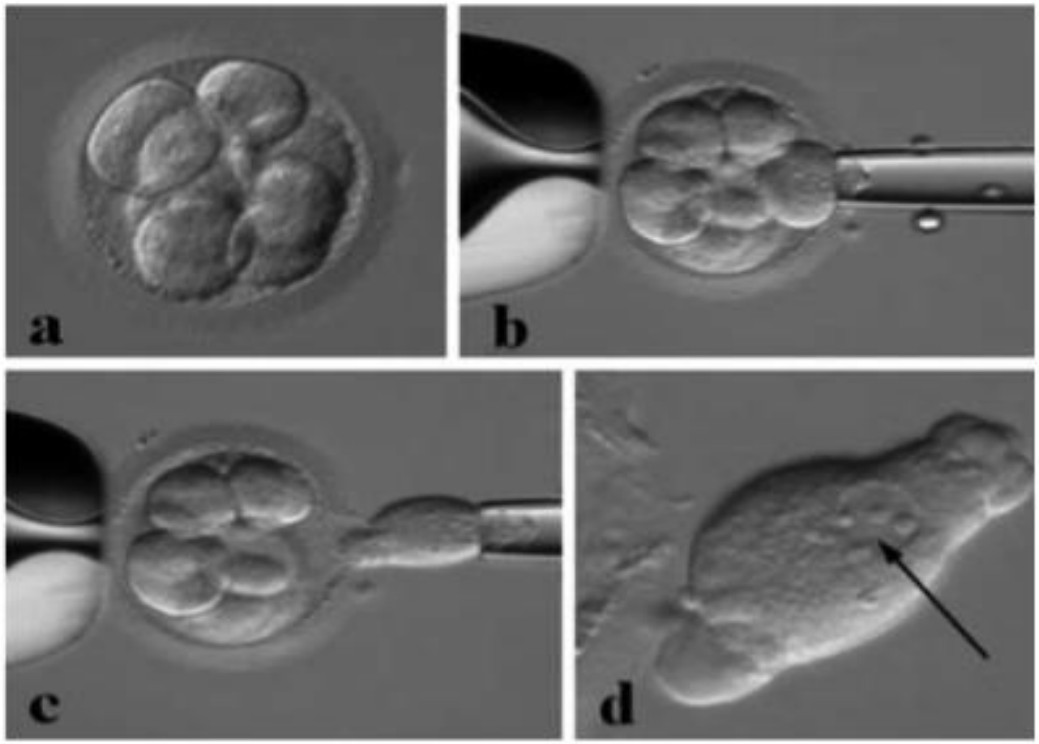
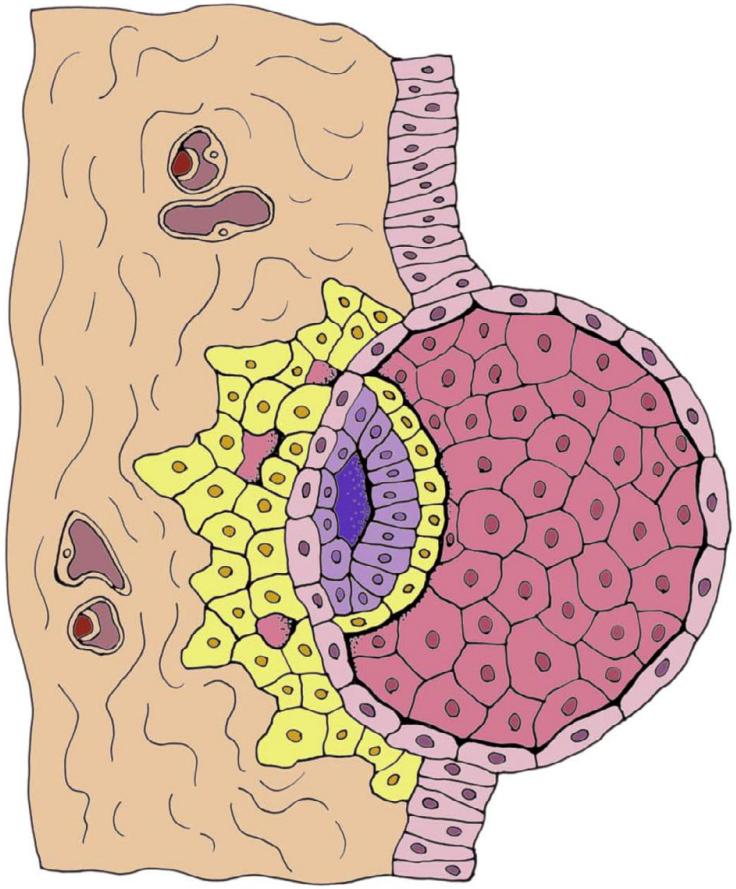


Figure 2 Blastomere biopsy of a human cleavage-stage embryo. (**a**) Eight-cell embryo, day 3 postfertilization; (**b**) embryo on holding pipette (left), with biopsy pipette (right) breaching the zona pellucida; (**c**) blastomere removal by suction; (**d**) biopsied blastomere with a clearly visible single nucleus (indicated by arrow). Some of these images have been published previously (Braude et al. 2002).

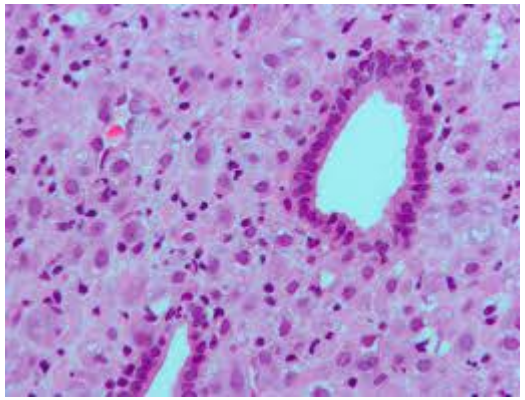
Challenges of PGS

- Still evolving
- Results conflicting
- Need advanced culture systems
- Need high skill

Oocyte+Sperm(Embryo)+Uterus= Implantation



Endometrium

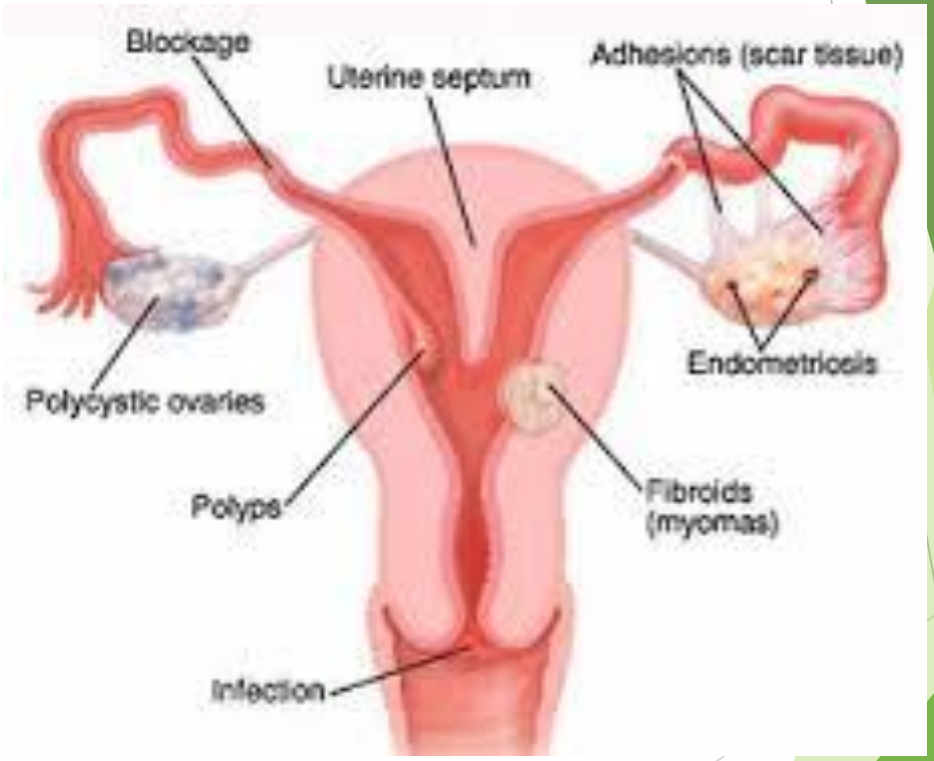


Endometrial Receptivity

- Depends upon
  - Uterine cavity abnormalities/pathologies
  - Endometrial thickness
  - Altered expression of adhesive molecules
  - Immunological factors

Uterine abnormalities/pathologies

- Uterine abnormalities
- Hyperplasia
- Polyps
- Endometritis
- Synechiae
- Leiomyoma

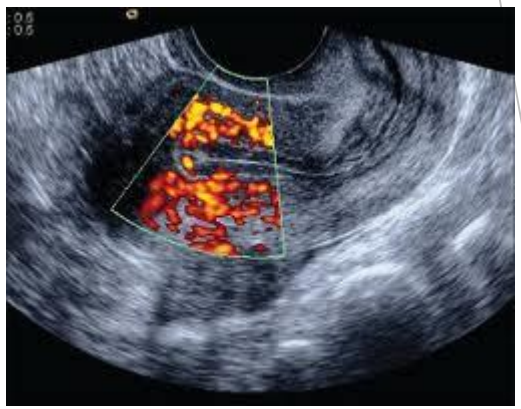


Investigations (Uterine & Other factors)

- USG pelvis – 2 D, 3 D
- HSG, SSG
- Endoscopy
  - Hysteroscopy
  - Laparoscopy
- Endometrial biopsy – Uterine NK cells, TNF α
- Endometrial Receptivity Array (ERA)

Endometrial Lining

- Endometrial thickness
- Endometrial volume
- Endometrial pattern
- Uterine artery blood flow
- Endometrial / subendometrial blood flow



Findings at Routine Hysteroscopy.

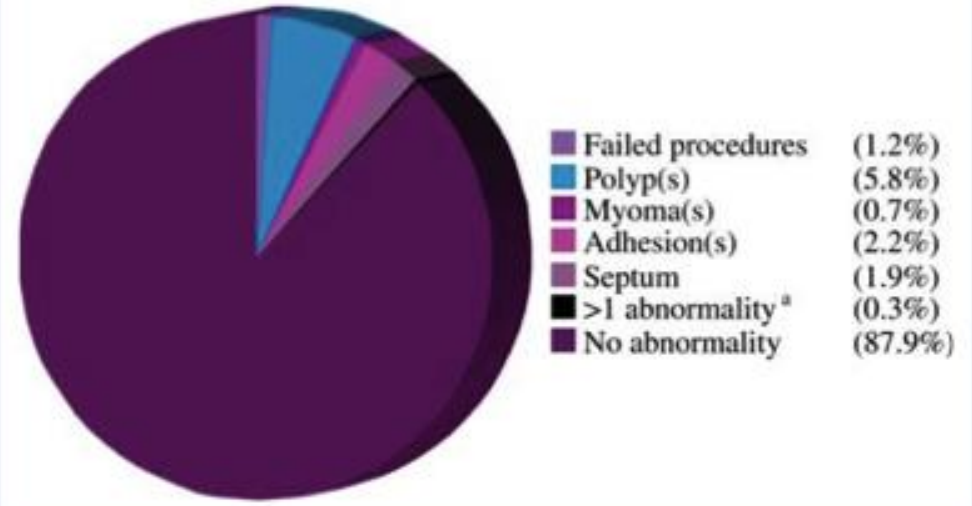


Figure 2 Findings at office hysteroscopy. [a]In one case both an endometrial polyp and a submucous myoma were diagnosed. In another case both an endometrial polyp and a septate uterus were diagnosed.

Implantation – A Complex Process !

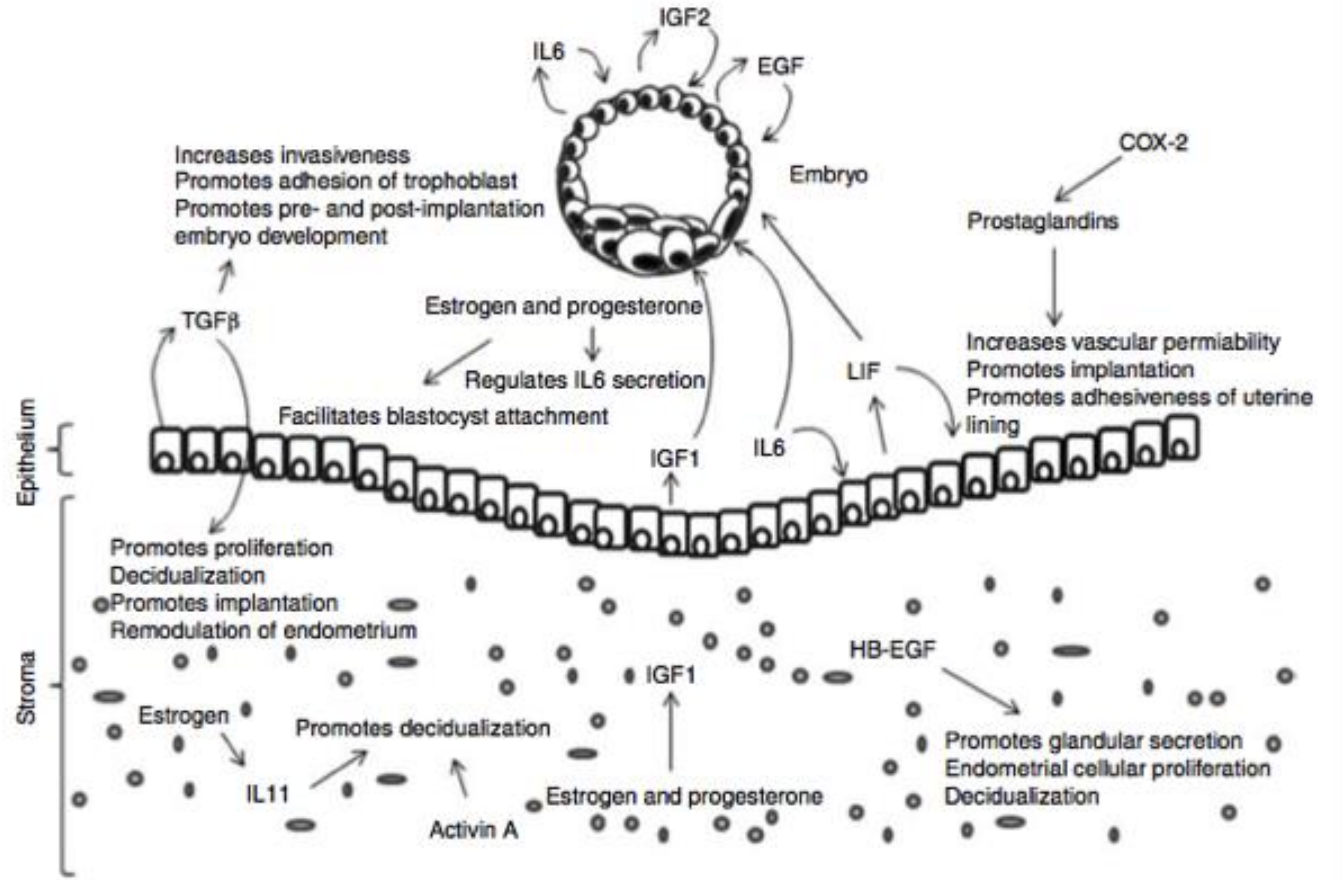
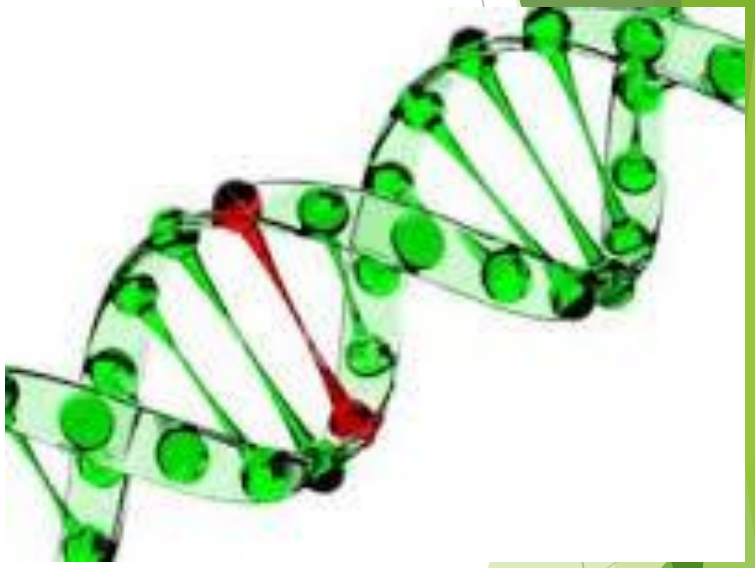


Figure 1 Summary of the various growth factors, cytokines, and hormones involved in implantation process.

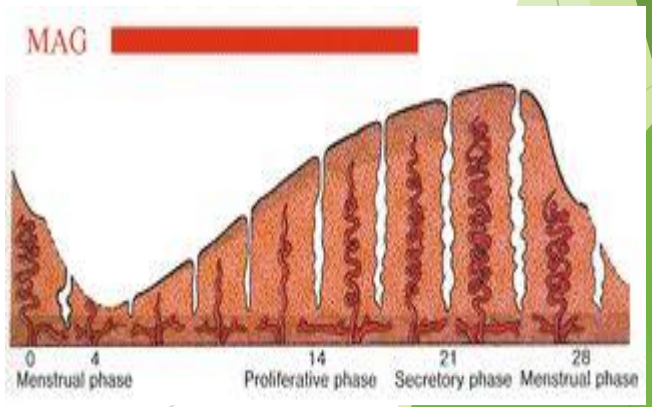
Immunological factors

- Natural Killer (NK) Cells – Uterine
- Cytotoxic Lymphocytes (CTLs)
- Antithyroid Antibodies (ATA)
- Alloimmune Implantation Dysfunction: HLA compatibility; HLA-G and DQ-alpha
- Antiphospholipid antibodies (lupus anticoagulant and the anticardiolipin antibodies)
- Autoimmune Response to Sperm Antigen



Endometrial Receptivity Array (ERA)

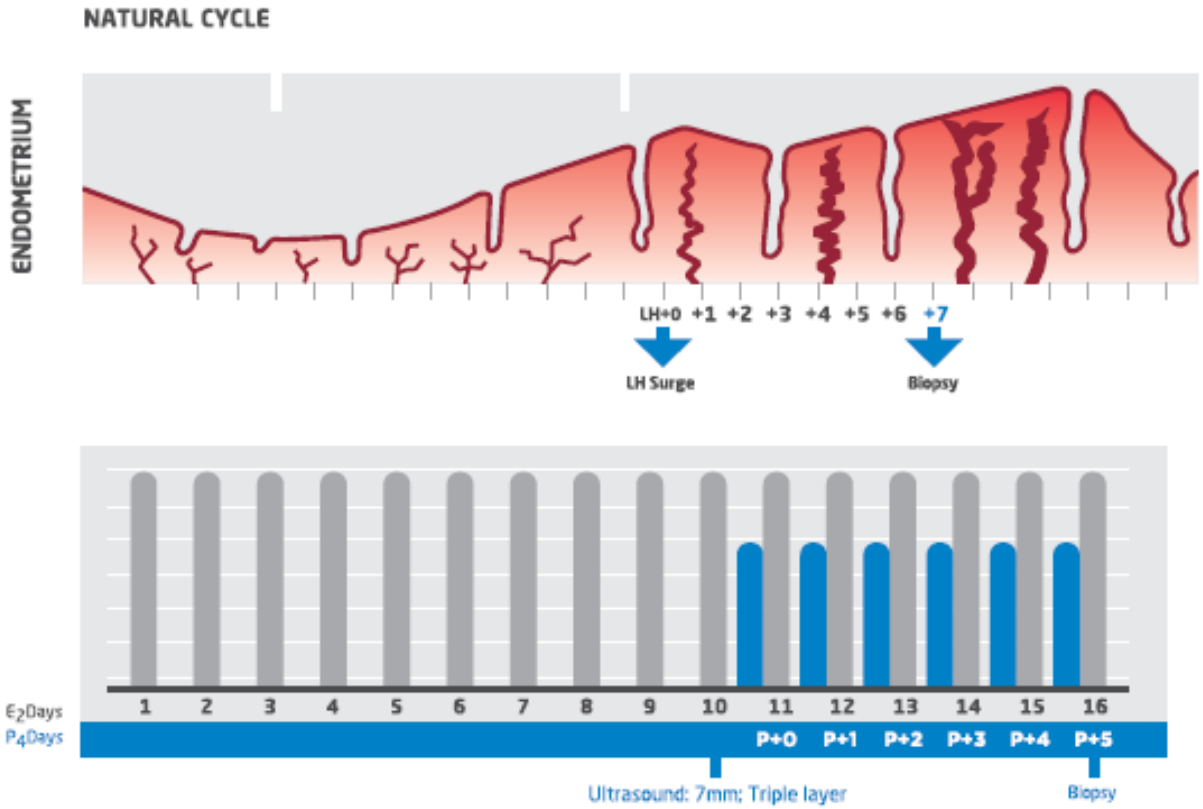
- Genetic test to diagnose state of endometrial receptivity in window of implantation
- Analyse expression levels of 238 genes related to status of endometrial receptivity
- Recommended for younger women with at least 3 failed embryo transfers or for patients 37 years or more with 2 failed embryo transfers

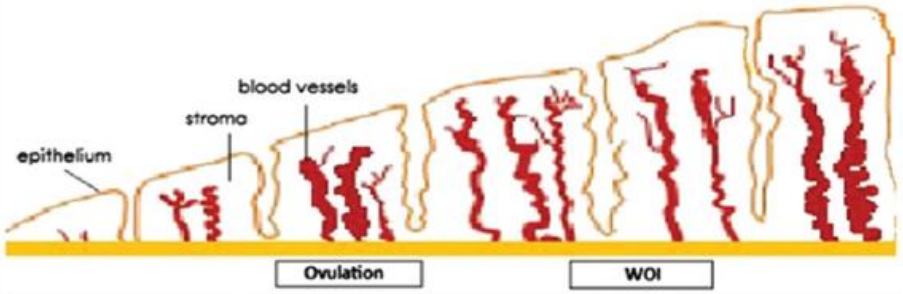


Endometrial Receptivity Array

- Endometrial biopsy –
  - Natural cycle at day 21 i.e 7 days after LH surge LH+7 or 6 days after the follicle rupture, when monitored by ultrasound)
  - Hormone replacement therapy cycle after 5 full days of progesterone impregnation in HRT cycles
- After biopsy - immediately introduced into an “ERA cryotube” containing fluid that allows preservation of tissue

Endometrial receptivity array





Proliferative	Pre-Receptive	Receptive	Post-Receptive
			
Proliferative functions	**Secretory functions**		
Cellular proliferation Cellular differentiation Extracellular matrix remodeling Angiogenesis and vasculogenesis DNA synthesis Adhesion Ion channels	*Early-secretory* Metabolism Transport Proliferation inhibition Mitosis inhibition	*Mid-secretory* Metabolism Glandular secretion Cell differentiation Cell communication Innate immune response Response to stress Response to wounding Adhesion Proteolysis regulation	*Late-secretory* Extracellular matrix degradation Inflammatory response Apoptosis

Interpretation of results

- **(R) Receptive :**
  - **Advised to proceed with ET in same conditions, type of cycle and day when EB has been done**
- **(NR) Non Receptive :**
  - **New EB to be taken to validate implantation window displacement and guide ET**

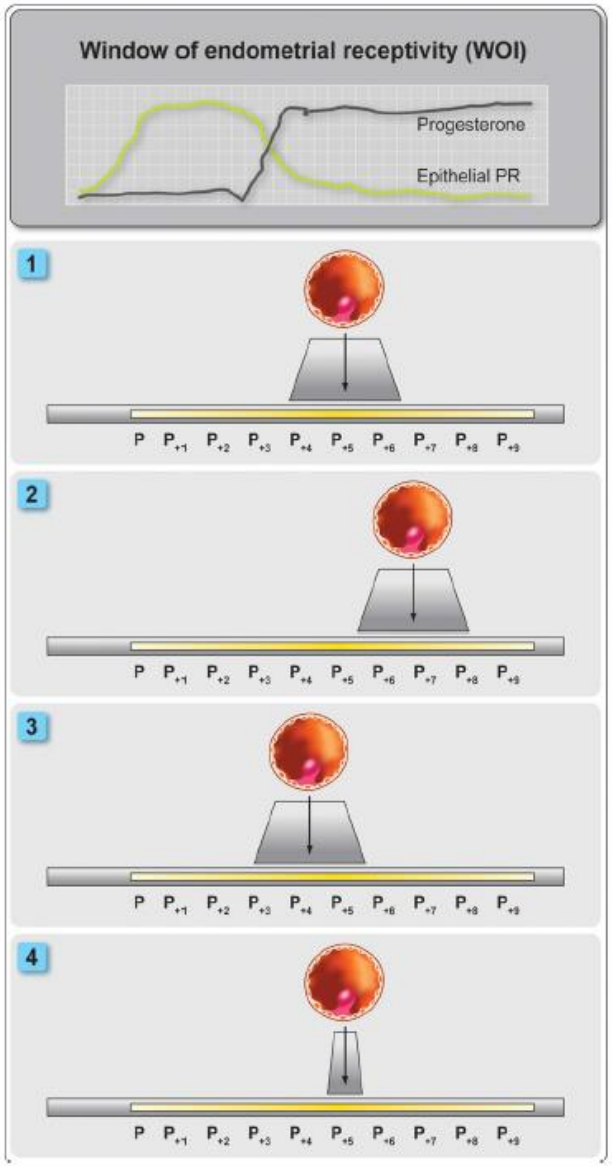


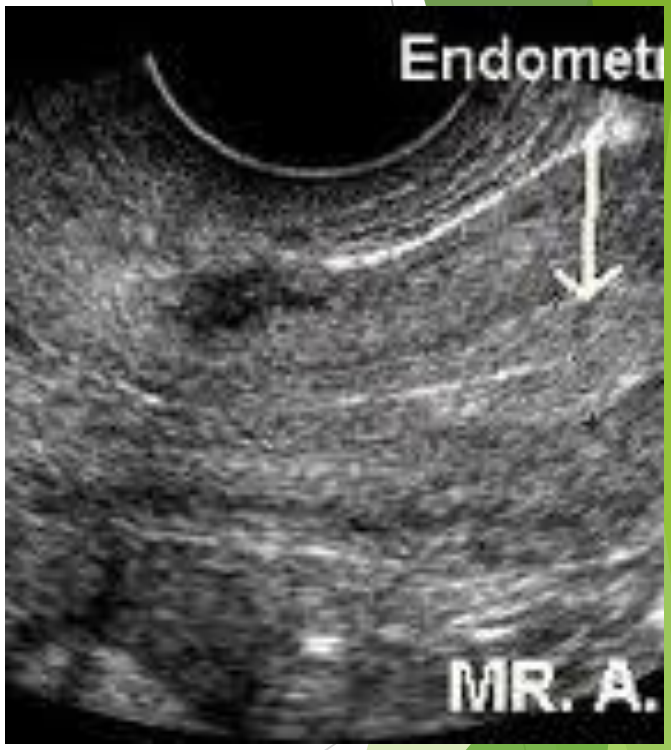
Figure 4 Displacement of the window of implantation (WOI). It has been assumed that the WOI is constant in time in all women (1). However, the genomic signature of the endometrium demonstrates the existence of a displacement of the WOI in up to 25% of patients that can be delayed (2), advanced (3) or shorter than expected (4). 'P_{+x}' refers to the days after progesterone administration.

Conclusion

- ERA can be helpful in patients with RIF
- Requirement of non invasive method of testing
- Individualizes ET timing
- Small numbers/ Retrospective
- Further research required
- Cost : benefit requires work

Improving Soil

- Hysteroscopic correction of cavity pathology
- Myomectomy
- Treatment of thin endometrium (High-dose estrogens, low-dose aspirin & vaginal sildenafil,arginine)
- Endometrial stimulation (biopsy) - pseudo-decidual reaction
- Immunotherapy (intravenous immunoglobulin, steroids, aspirin and heparin)
- Anti – Tubercular Therapy
- Treating Adenomyosis
- Changing day of transfer



Aspirin Study

Cochrane Database Syst Rev. 2009 Jan 21;(1):CD004734. doi: 10.1002/14651858.CD004734.pub3.

Aspirin or anticoagulants for treating recurrent miscarriage in women without antiphospholipid syndrome.

Kaandorp S[1], Di Nisio M, Goddijn M, Middeldorp S.

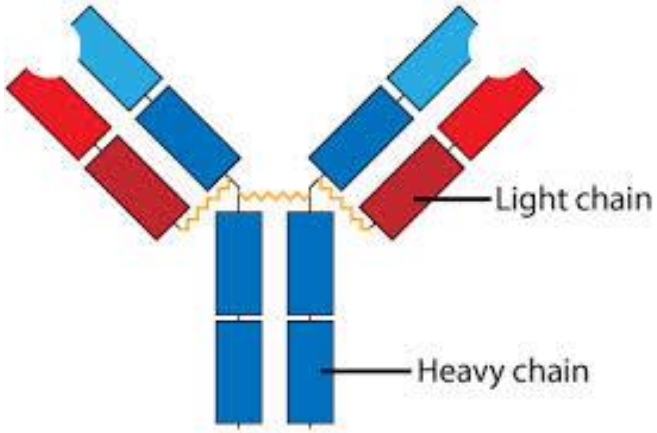
AUTHORS' CONCLUSIONS: There is a paucity in studies on the efficacy and safety of aspirin and heparin in women with a history of at least two miscarriages without apparent causes other than inherited thrombophilia. The two reviewed trials studied different treatments and only one study was placebo-controlled. Neither of the studies showed a benefit of one treatment over the other. Therefore, the use of anticoagulants in this setting is not recommended. However, large randomised placebo-controlled trials are still urgently needed.

Heparin

MOA

- Anticoagulant
- Immunomodulatory
- Anti-inflammatory effect

- Results in adhesion of blastocyst to endometrial epithelium and subsequent invasion



Prednisolone

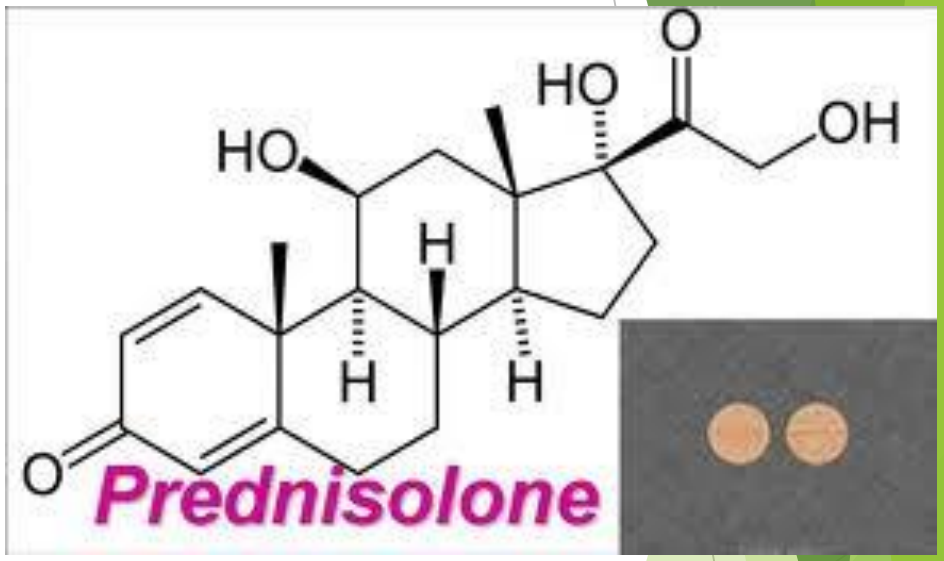
MOA

- Immunosuppression
  - ⬇ uterine NK cells
  - ⬇ Cytokines
  - ⬇ Endometrial inflammation

⬇

Better implantation rate

- Orally
- 10 mg once daily (Before breakfast



Sildenafil

MOA

- Selective inhibitor of the type V cGMP- specific phosphodiesterase

↓

↑Vasodilatory effects of nitric oxide

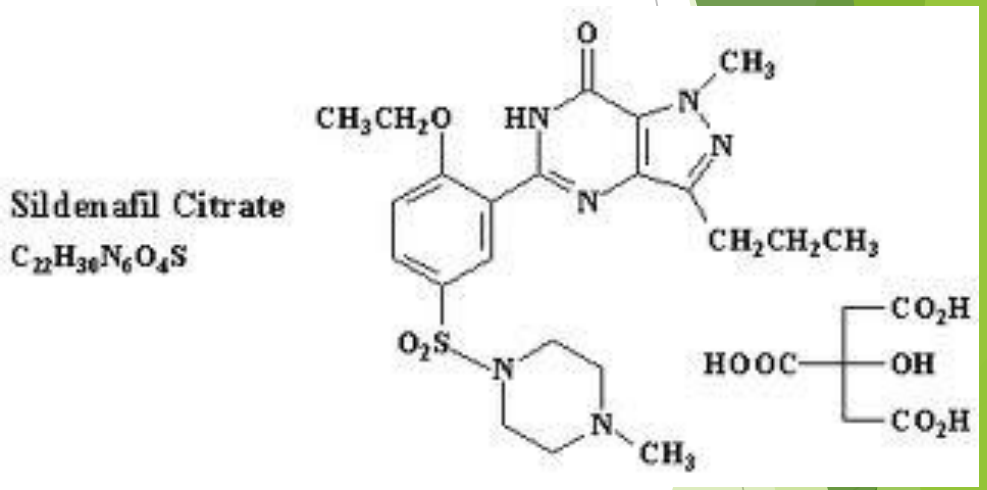
↓

↑Uterine blood flow

↓

Improved endometrial thickness

- 25 mg once in night (Vaginally)



Am J Reprod Immunol. 2013 Dec;70(6):434-47. doi: 10.1111/aji.12170.

Full Text Online Library

Intravenous immunoglobulin treatment for repeated IVF/ICSI failure and unexplained infertility: a systematic review and a meta-analysis.

Li J[1], Chen Y, Liu C, Hu Y, Li L.

per embryo transferred was not (2.893; 95%CI: 0.810-10.331) less. Our results strongly support that IVIG is a useful treatment option for women undergoing repeated IVF failure.

THE EFFECT OF INTRALIPID ON PREGNANCY RATES IN *IN VITRO* FERTILISATION (IVF).

R. H. Shirlow,[a] M. Healey,[b,c,d] M. Volovsky,[a] V. B. MacLachlan,[d] B. J. Vollenhoven.[e] [a]Monash University,

CONCLUSIONS: Intralipid has been introduced to improve pregnancy rates in women with implantation failure. Based on these results there is no difference in outcomes once confounding factors have been allowed for. Our study concludes that, at present, intralipid should not be advocated for as a beneficial therapy and future use should only be as part of a randomised controlled trial. The safety of intralipid also needs investigation.

http://dx.doi.org/10.1016/j.fertnstert.2016.07.956

Consensus re ATT

- AFB Pos
- IGRA test + PCR Positive
- Clinical doubt of TB

- Interferon Gamma Release Assay
- Nucleic Acid Amplification Test

Asif et al., J Women's Health Care 2014, 3:6
http://dx.doi.org/10.4172/2167-0420.1000207

Women's Health Care

Research Article Open Access

Adenomyosis and Its Effect on Reproductive Outcomes

Sonia Asif*, Ian Henderson and Nick Raine-Fenning

Conclusion: Further epidemiological studies are needed to evaluate the prevalence and impact of adenomyosis in the sub-fertile population. Currently the existing literature on the effect of the disease on fertility is inconclusive; hence, actively diagnosing and treating the condition is debateable in women wishing to conceive. However, once pregnancy is achieved, the evidence suggests a detrimental effect on delivery rate, with an increased risk of miscarriage and preterm labour. There is some success seen in treating women undergoing assisted reproduction. Clinicians could consider a long course of pituitary down regulation prior to ART in appropriately informed sub-fertile women.

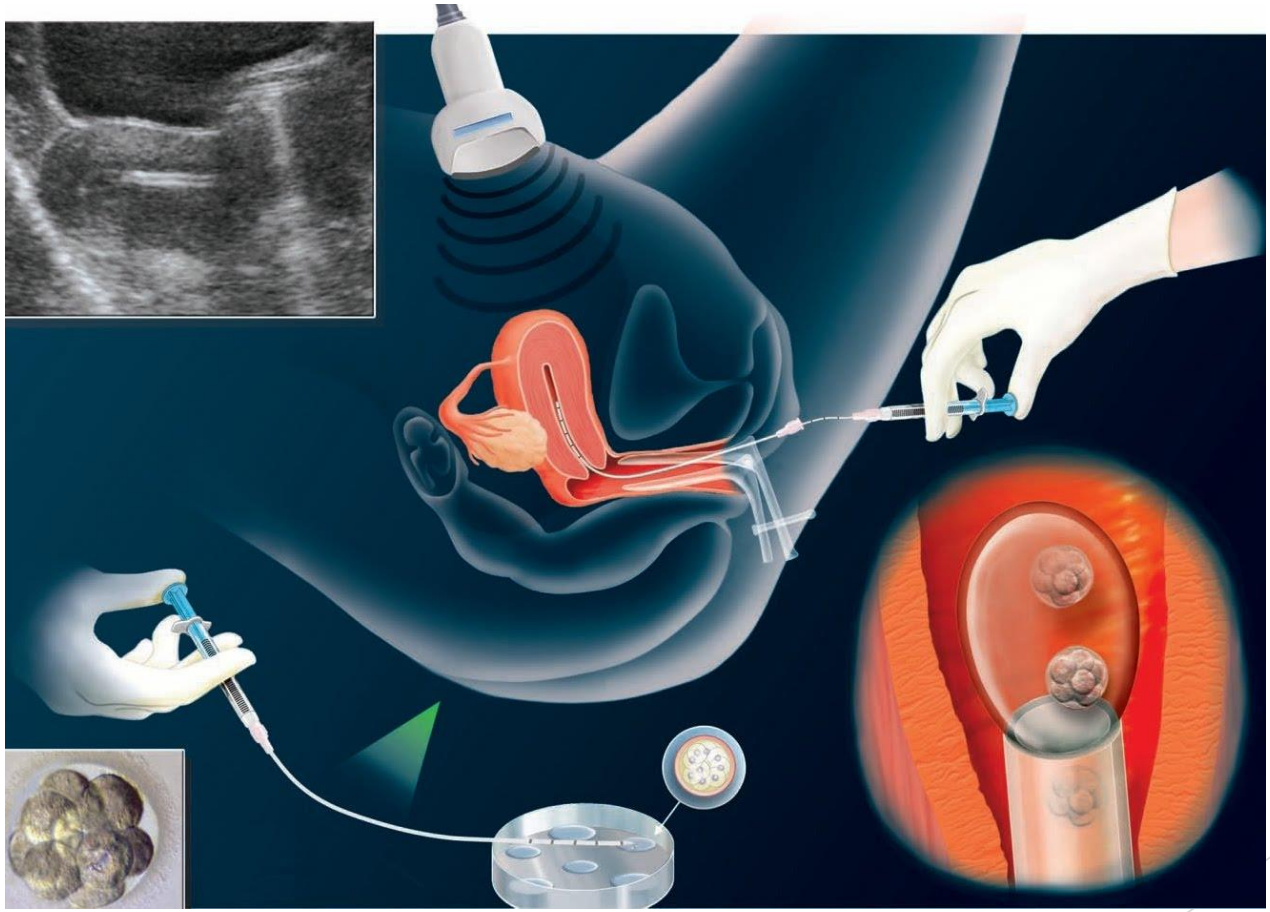
Other Factors

- Hydrosalpinges
- Embryo transfer techniques
- Adjunctive treatment (Metformin, Bromocriptine, Thyroxine)
- Alternative treatment

Embryo ---------- Uterus

The Missing Link

Embryo Transfer




MAXIMAL IMPLANTATION POTENTIAL

Improving ET technique

- USG guided
- Mock ET
- Full bladder
- Use of Stylet
- Irrigation and aspiration of cervical mucus
- Maximal Implantation Point

Sequential Transfer

- D2/D3 and D5
- Same Cycle
- RIF
- Freezing not option
- Multiple Pregnancy

Correct Cavity

Treat Hydrosalpinx

Hysteroscopy & Endometrial Scratching

Improve Protocol

Delayed Transfer

IVIG

Assisted hatching

Sequential Transfer

LMWH

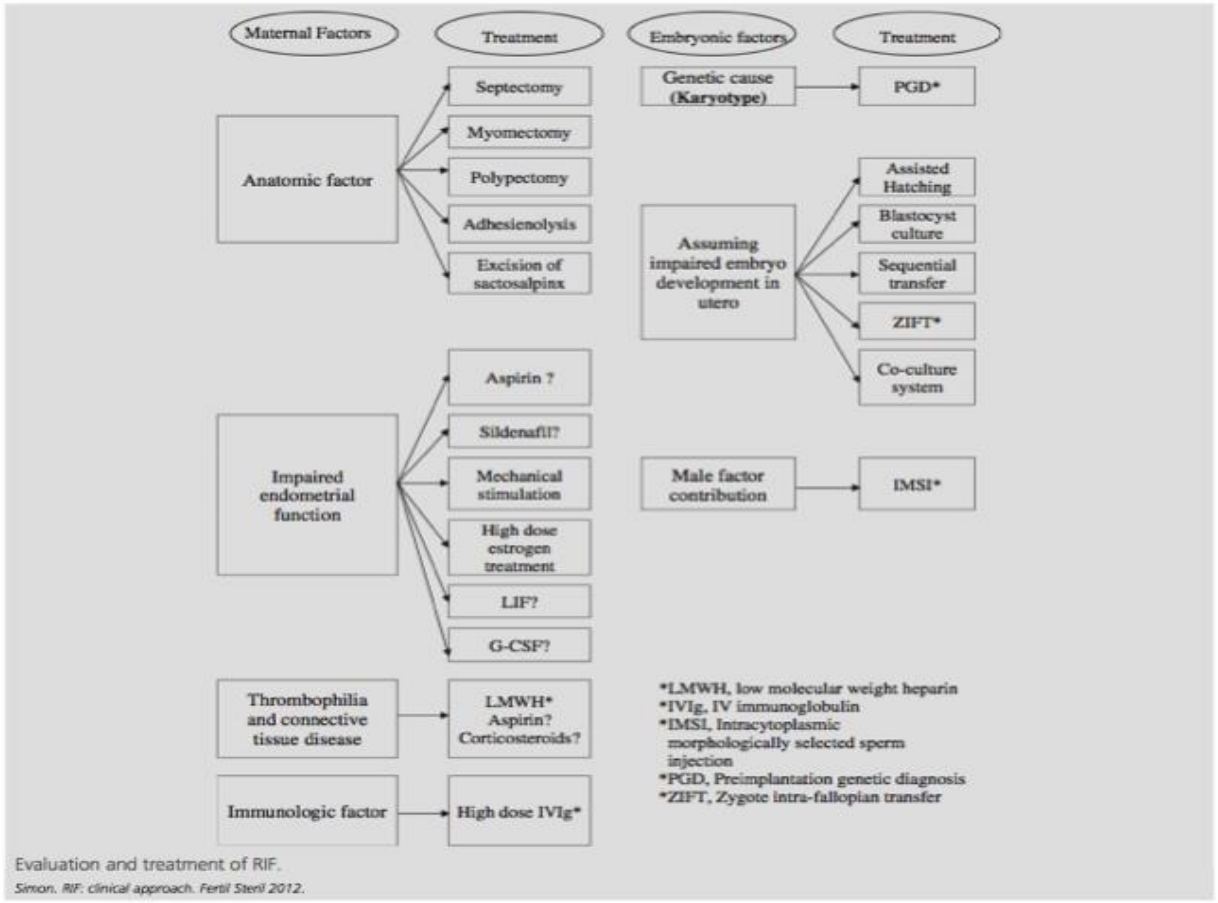
PGS

J Assist Reprod Genet (2012) 29:1227–1239
DOI 10.1007/s10815-012-9861-4

ASSISTED REPRODUCTION TECHNOLOGIES

Assessment and treatment of repeated implantation failure (RIF)

Alex Simon · Neri Laufer



Evaluation and treatment of RIF.
Simon. RIF: clinical approach. Fertil Steril 2012.

Reproductive BioMedicine Online (2014) **28**, 14–38

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REVIEW

Recurrent implantation failure: definition and management

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Dr Carol Coughlan received her medical training in Ireland and obtained her MRCOG in 2003 and MRCPI in 2004. She completed subspecialist training in reproductive medicine and surgery at the Jessop Wing, Royal Hallamshire Hospital, Sheffield, UK. She is pursuing her research interests in recurrent implantation failure and recurrent miscarriage.

Human Reproduction Vol.21, No.12 pp. 3036–3043, 2006 doi:10.1093/humrep/del305
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Mini Review—Developments in Reproductive Medicine

Investigation and treatment of repeated implantation failure following IVF-ET

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Thank you

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