
# ART and miscarriage risk

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# Miscarriage rate

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- 10 % to 15 % of clinical pregnancies have resulted in miscarriage.

- 1-2% miscarriages / per couples who try to conceive.

(Macklon NS et al, 2002; Rai R  et al 2006)

# Monthly fecundity rate (MFR)

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- In humans even in optimal circumstances – clinical recognized pregnancy in one cycle or the so called monthly fecundity rate is around 30 % .

- In contrast MFR is 80% in baboons and 90% in rabbits.

(Chard T, 1991; . Foote RH 1988; Stevens VC 1997)

# Ongoing pregnancy rate

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- Assisted reproductive technologies (ART) represent average 30 % pregnancy rate.
- Around 50% of human conception fails implantation.
- Up to half of implanted embryos fail to progress in ongoing pregnancy.
  - (Macklon N 2002; Macklon N 2014)

# Conception to ongoing pregnancy

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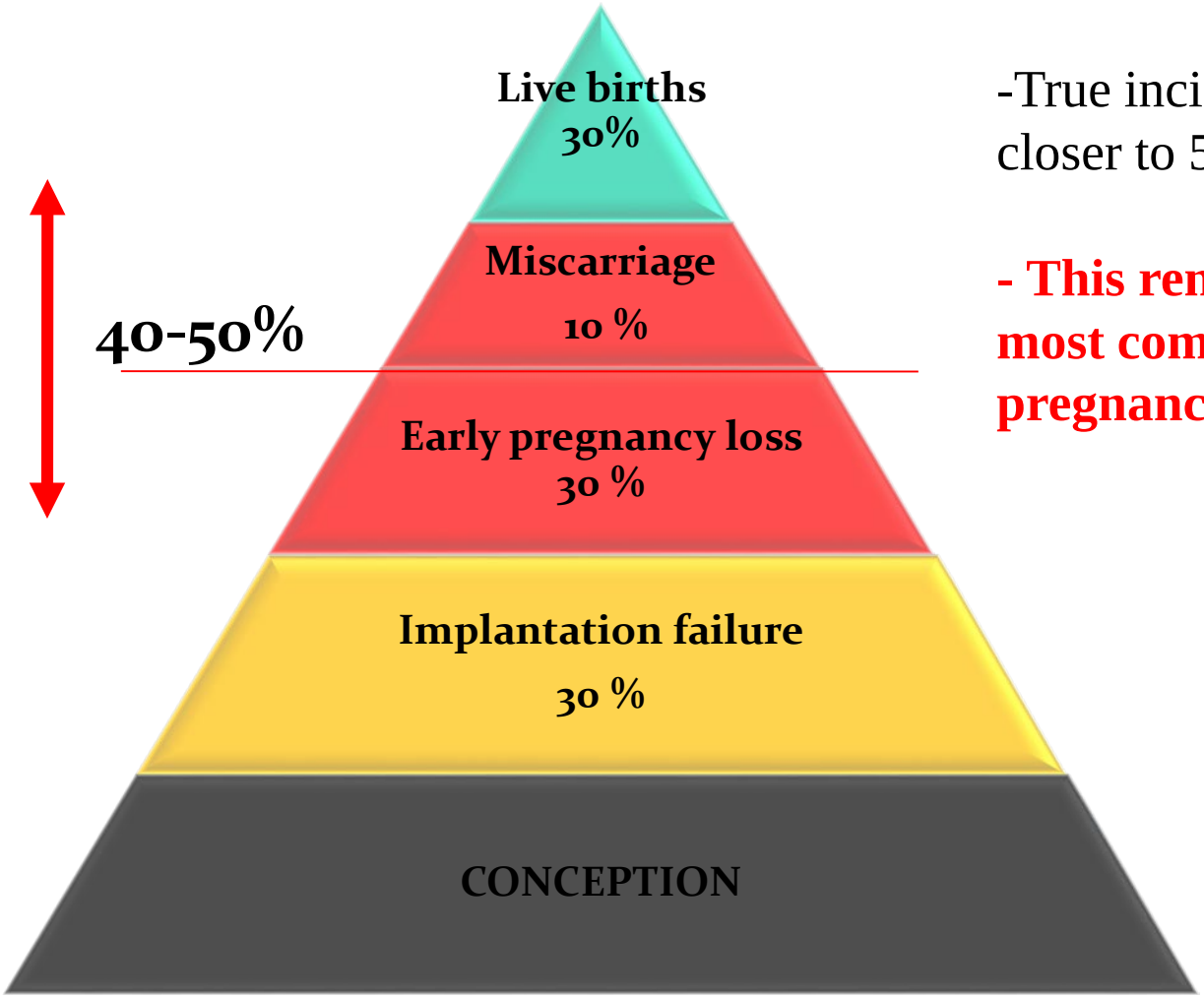


-True incidence of pregnancy loss is closer to 50%.

**- This renders miscarriage as the most common complication of pregnancy**

Macklon et al, Hum Reproduction Update, 2002

# Known reasons for miscarriage

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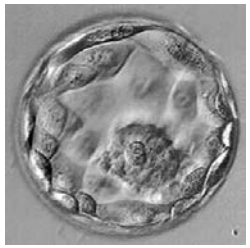
- Antiphospholipid syndrome
- Endocrine abnormalities
  - Thyroid dysfunction
  - Diabetes
- Chromosome aberrations
- Uterine structural malformation
- Trombophilias
- Unknown factors in 50 % of cases.

# Embryo



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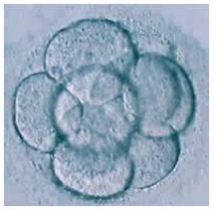
- The enormous rate of early pregnancy loss in humans thought to be as a consequences of two key features of human embryos:

- 1. High prevalence of **chromosomal abnormalities.**
- 2. Invasiveness.

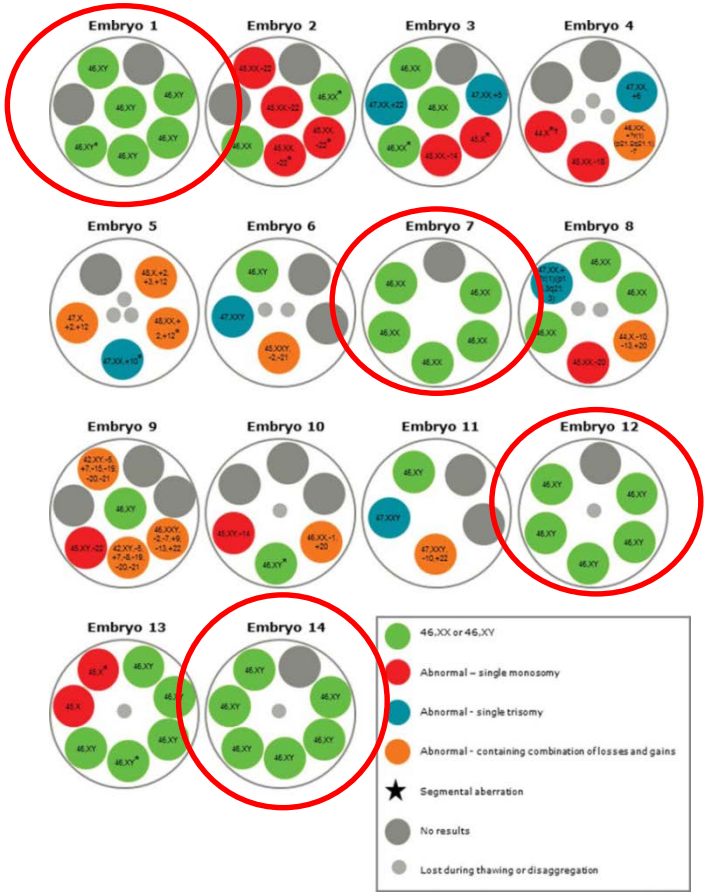
Human Reproduction, Vol.28, No.1 pp. 256–264, 2013
Advanced Access publication on October 9, 2012 doi:10.1093/humrep/des362

human reproduction ORIGINAL ARTICLE *Reproductive genetics*



# Microarray analysis reveals abnormal chromosomal complements in over 70% of 14 normally developing human embryos

A. Mertzanidou[1,†], L. Wilton[2,†], J. Cheng[3,4,†], C. Spits[1], E. Vanneste[5], Y. Moreau[3,4], J.R. Vermeesch[5], and K. Sermon[1,*]




-Good-quality cleavage-stage embryos exhibit high rates of aneuploidy.

-28.6% - completely normal.

-71.4% - mosaic and structural aberrations.

- No embryo contained the same aneuploidy in all of its cells.

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# Aneuploidy of the embryo

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- The rate of aneuploidy is decreased during development to the blastocyst stage.
  - Santos MA et al, Hum Reproduction 2010:
    - Prevalence of aneuploidy decreases from 83% on Day 4 of development to 42% on Day 8.
  - Fragouli E et al , Hum Genet 2013:
    - **Observed aneuploidies in 58% of blastocysts;**
    - **Peri-implantation human embryos are intrinsically chromosomally diverse and predominantly mosaic.**

# Aneuploidy of the embryo

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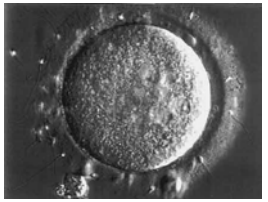
- Preimplantation genetic diagnosis (PGD) reveals that women with recurrent miscarriage (RM) produce more aneuploid embryos than normal women

  (Simon 1998, Vidal 1998, Pellicer 1999, Gianaroli 2000).

- The majority of these aneuploid embryos come from maternal oocyte abnormalities

  (Cobo 2001, Robinson 2001)

# Oocyte abnormalities



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- Ovarian hyperstimulation - risk factor for high rate of oocyte abnormality

  (London et al 2000; Santos MA, 2010; Baart et al. 2007; Katz-Jaffe et al. 2005; Lubbadeh et al. 1980, Kanayama & Osada 2000).

- Diminished ovarian reserve (DOR) as a cause of recurrent miscarriage

# Diminished ovarian reserve: is it a neglected cause in the assessment of recurrent miscarriage? A cohort study

Fertility and Sterility, May 2016

Melahat Atasever, M.D.,[a] Zeynep Soyman, M.D.,[b] Emine Demirel, M.D.,[c] Servet Gencdal, M.D.,[c] and Sefa Kelekci, M.D.[c]

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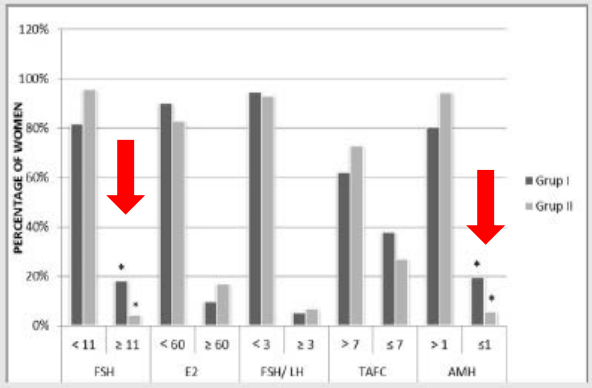
**Comparison of demographic characteristics and ovarian reserve test parameters between recurrent miscarriage and control groups.**

| Parameter | Recurrent miscarriage (n = 71) | Control (n = 70) | *P* value |
|---|---|---|---|
| Age (y) | 29.5 ± 4.5 | 29.1 ± 4.7 | NS |
| ≤30 | 42 (59.2%) | 43 (61.4%) | NS |
| >30 | 29 (40.8%) | 27 (38.6%) | |
| BMI (kg/m$^2$) | 24 ± 3.2 | 25 ± 3.9 | NS |
| Mean cycle length (d) | 28.3 ± 2.2 | 28.5 ± 1.5 | NS |
| Gravidity | 3.7 ± 0.9 | 1.7 ± 0.6 | .001 |
| Parity | 0.2 ± 0.4 | 1.5 ± 0.7 | .001 |
| Pregnancy loss | 3.5 ± 0.9 | 0.09 ± 0.2 | .001 |
| FSH (U/L) | 8.6 ± 3.7 | 7.1 ± 1.9 | .049 |
| FSH ≥11 U/L | 13 (18.3%) | 3 (4.3%) | .009 |
| LH (U/L) | 5.2 ± 2.2 | 5.1 ± 2.4 | NS |
| $E_2$ (nmol/L) | 42.2 ± 15.1 | 45.5 ± 30.2 | NS |
| $E_2$ ≥60 nmol/L | 7 (9.9%) | 12 (17.1%) | NS |
| FSH/LH | 1.7 ± 0.7 | 1.6 ± 1.1 | NS |
| FSH/LH ≥3 | 4 (5.6%) | 5 (7.1%) | NS |
| ROV (mL) | 6.0 ± 2.3 | 6.1 ± 1.7 | NS |
| LOV (mL) | 6.1 ± 2.2 | 6.0 ± 1.7 | NS |
| MOV (mL) | 6.0 ± 2.0 | 6.1 ± 1.6 | NS |
| ROAFC (n) | 4.9 ± 2.0 | 5.0 ± 2.0 | NS |
| LOAFC (n) | 5.1 ± 2.2 | 4.7 ± 2.0 | NS |
| TAFC (n) | 9.4 ± 4.0 | 9.8 ± 3.8 | NS |
| TAFC ≤7 | 27 (38%) | 19 (27.1%) | NS |
| AMH (ng/mL) | 2.9 ± 1.7 | 3.6 ± 1.7 | .007 |
| AMH ≤1 ng/mL | 14 (19.7%) | 4 (5.7%) | .013 |

*Note:* Results are presented as mean ± SD or n (%). AMH = antimüllerian hormone; BMI = body mass index; LOAFC = left ovary antral follicle count; LOV = left ovarian volume; MOV = mean ovarian volume; NS = not significant; ROAFC = right ovary antral follicle count; ROV = right ovarian volume; TAFC = total antral follicle count.

*Atasever. Ovarian reserve in recurrent miscarriage. Fertil Steril 2016.*




Percentage of women with diminished ovarian reserve in the recurrent miscarriage and control groups. TAFC = total antral follicle count; AMH = antimüllerian hormone.

*Atasever. Ovarian reserve in recurrent miscarriage. Fertil Steril 2016.*

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Gynecology and Obstetrics
July 2004, Volume 270, Issue 1, pp 37–39

# High basal estradiol level and FSH/LH ratio in unexplained recurrent pregnancy loss

Authors

Birgül Gürbüz  Serap Yalti, Selcuk Ozden, Cem Ficicioglu

**Unexplained RPL may be associated with diminished ovarian reserve and should be considered in the workup of RPL.**

# Do women with unexplained recurrent pregnancy loss have higher day 3 serum FSH and estradiol values?

Fertill Steril, 2000

*Susan W. Trout, M.D., and David B. Seifer, M.D.*

*Division of Reproductive Endocrinology and Infertility, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, New Jersey*

**Diminished ovarian reserve may contribute to recurrent pregnancy loss**

Obstetrics & Gynecology:
January 2013 - Volume 121 - Issue 1 - p 71–77
doi: http://10.1097/AOG.0b013e318278eeda
Original Research

# Association of Abnormal Ovarian Reserve Parameters With a Higher Incidence of Aneuploid Blastocysts

Katz-Jaffe, Mandy G. PhD; Surrey, Eric S. MD; Minjarez, Debra A. MD; Gustofson, Robert L. MD; Stevens, John M. BS; Schoolcraft, William B. MD

**Infertility patients with hormonal evidence of diminished ovarian reserve have a significantly higher percentage of aneuploid blastocyst**

# Higher rates of aneuploidy in blastocysts and higher risk of no embryo transfer in recurrent pregnancy loss patients with diminished ovarian reserve undergoing in vitro fertilization

Lora K. Shahine, M.D., Lorna Marshall, M.D., Julie D. Lamb, M.D., and Lee R. Hickok, M.D.
Pacific NW Fertility, Seattle, Washington

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**Cycle characteristics**

| Characteristic | Normal ovarian reserve (n = 59) | DOR (n = 43) | *P* value |
|---|---|---|---|
| Total amount of gonadotropins (IU) | 3,204.5 ± 1,124.5 | 4,432.5 ± 1,562.4 | .04 |
| No. of mature eggs | 12.9 (8–33) | 8.5 (5–10) | .01 |
| No. of blasts biopsied | 5.3 (1–10) | 3.6 (2–6) | .02 |
| Aneuploid blasts (%) | 48 | 57 | .03 |
| % All aneuploid blasts | 13 | 25 | .02 |

*Note:* Values are averages unless otherwise noted.

*Shahine. High aneuploidy in RPL patients with DOR. Fertil Steril 2016.*

Fertility and Sterility, Oct. 2016

# Higher rates of aneuploidy in blastocysts and higher risk of no embryo transfer in recurrent pregnancy loss patients with diminished ovarian reserve undergoing in vitro fertilization

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Pacific NW Fertility, Seattle, Washington

Fertility and Sterility, Oct 2016

**Percentage of aneuploid embryos according to age.**

| Age (y) | Normal ovarian reserve (n = 59) | DOR (n = 43) | *P* value |
|---|---|---|---|
| <35 | 45 | 59 | .04 |
| 35–37 | 59 | 77 | .03 |
| 38–40 | 74 | 76 | .9 |
| 41+ | 87 | 92 | .8 |

*Note:* Values are percentages.

*Shahine. High aneuploidy in RPL patients with DOR. Fertil Steril 2016.*

1. Performing IVF with CS in RM patients

2. Ovarian reserve testing for RPL patients

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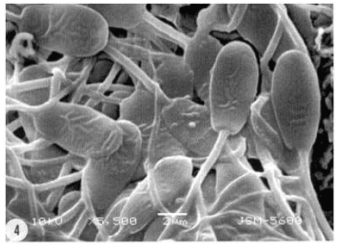
# Role of spermatozoa in the etiology of miscarriage



**Although the most common cause of sporadic miscarriage is embryonic aneuploidy, primarily derived from aneuploid eggs, the question is what role the male partner might play in pregnancy loss.**

**Human Reproduction, Vol.27, No.10 pp. 2908–2917, 2012**
Advanced Access publication on July 12, 2012 doi:10.1093/humrep/des261

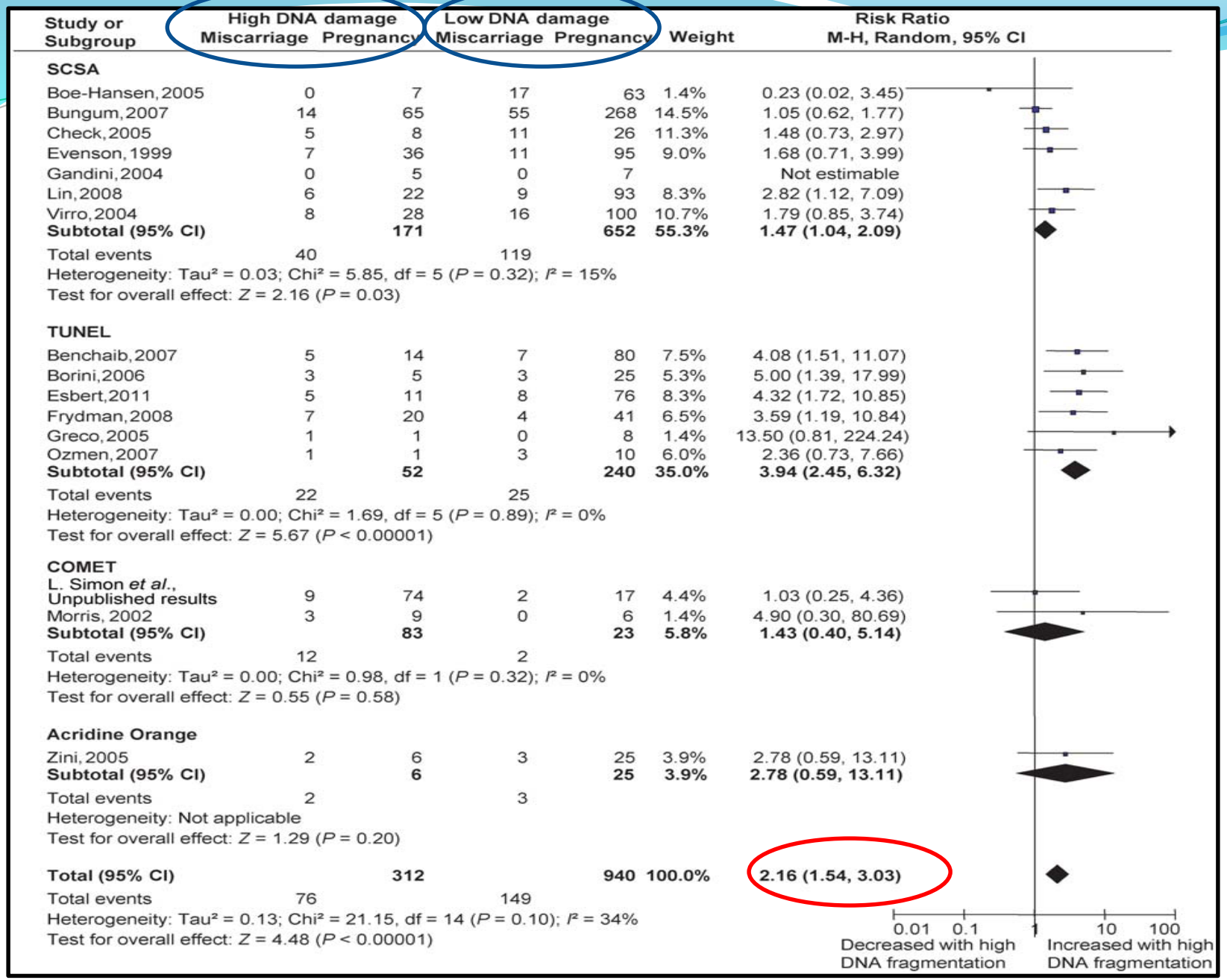
human reproduction

META-ANALYSIS *Andrology*



# The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis

**Lynne Robinson[1,*], Ioannis D. Gallos[1,2], Sarah J. Conner[1,2], Madhurima Rajkhowa[1], David Miller[3], Sheena Lewis[4], Jackson Kirkman-Brown[1,2], and Arri Coomarasamy[1,2]**




Robinson L et al; Hum Reprod 2012

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# Sperm DNA fragmentation and miscarriage

- The negative impact of high DNA fragmentation on pregnancy can be overcome by using high-quality oocytes (Meseguer M et al, 2008)

- There is a threshold beyond which sperm DNA damage cannot be repaired.

  - Lin M et al find out that in high DNA stainability ( HDS) > 15% and DNA fragmentation index (DFI) > 27 % can be expected higher abortion rate.

    (Lin M et al, 2008)

# Esteves S. et al; Fertil Steril 2015

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**Clinical pregnancy, miscarriage, and live birth in TESTI-ICSI and EJA-ICSI cohorts.**

| Variable | TESTI-ICSI (n = 77)[a] | EJA-ICSI (n = 87)[a] | *P* value | Relative risk (95% CI) |
|---|---|---|---|---|
| Embryos | | | | |
| Number, mean ± SD | 7.0 ± 3.7 | 6.4 ± 3.7 | .327 | NA |
| High quality on day 3 (%), mean ± SD | 45.2 ± 12.0 | 41.8 ± 14.1 | .118 | |
| No. transferred, mean ± SD | 2.0 ± 0.3 | 1.9 ± 0.6 | .206 | |
| Clinical pregnancy, n (%) | 40 (51.9) | 35 (40.2) | .131 | 1.29 (0.92–1.80) |
| Miscarriage, n (%) | 4 (10.0) | 12 (34.3) | .012 | 0.29 (0.10–0.82) |
| Live birth, n (%) | 36 (46.7) | 23 (26.4) | .007 | 1.76 (1.15–2.70) |

*Note:* NA = not applicable.

[a] Among the enrolled patients (see Table 1), a total of eight fresh transfers were cancelled, including four in the TESTI-ICSI group and four in the EJA-ICSI group.

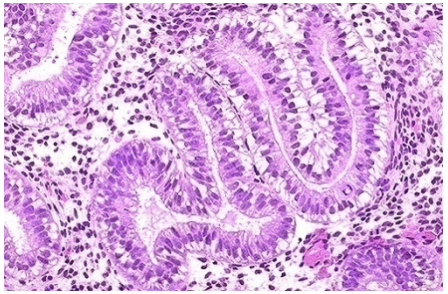
*Esteves. TESTI-ICSI in men with high SDF. Fertil Steril 2015.*

- A prospective, observational, cohort study in men with Olygoastenozoospermia and High sperm DNA Fragmantation (SDF)

- SDF index was approximately 5-fold lower in testicular sperm (TESTI) compared with in ejaculated sperm (EJA)

- Pregnancy and miscarriage rates favored the TESTI-ICSI group

# Endometrium



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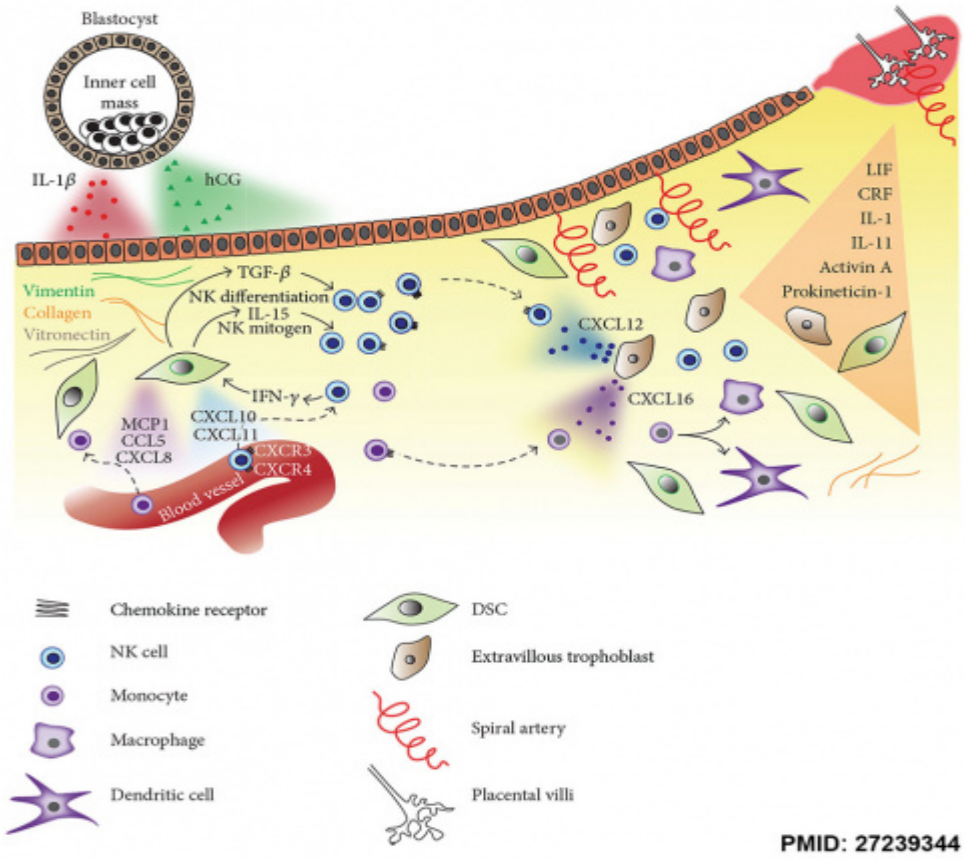
Achieving of pregnancy is depend on intimate interactions between a developmentally competent embryo and receptive endometrium.

# Decidua and implantation

- Endometrial decidualization is a process imperative for pregnancy
  - (transformation of stromal fibroblast into secretory decidual cells) (Gellersen B, 2007].
- Implantation depends on intimate dialogue between endometrial stromal cells (ESCs) and an interacting embryo
  - elicited by influx of specialized uterine immune response and vascular remodelling.
- **Decidua is a very important determinant for the selection of genomically abnormal blastocysts.**



# Decidula cells and impaired embryo

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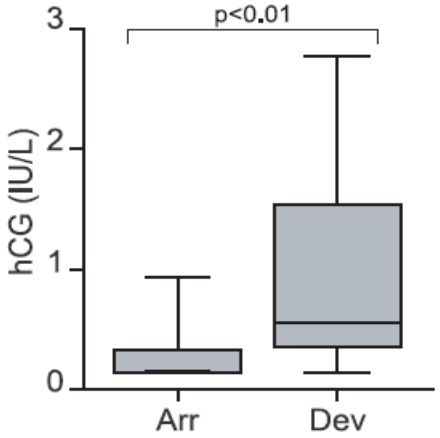
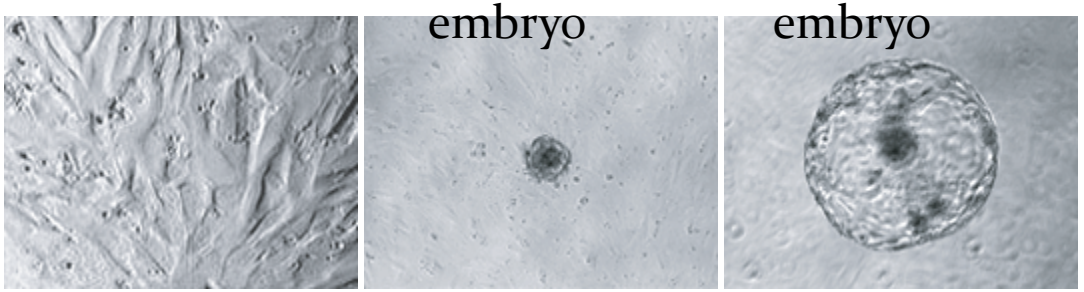
- Decidual cells are programmed to select against embryos with insufficient hCG production and by profound immune response (Teklenburg G et al,2010).




Arrested embryo

Developing embryo



# Natural Selection of Human Embryos: Decidualizing Endometrial Stromal Cells Serve as Sensors of Embryo Quality upon Implantation

Gijs Teklenburg[1,8], Madhuri Salker[2], Mariam Molokhia[3], Stuart Lavery[2], Geoffrey Trew[2], Tepchongchit Aojanepong[2], Helen J. Mardon[4], Amali U. Lokugamage[5], Raj Rai[2], Christian Landles[2], Bernard A. J. Roelen[6], Siobhan Quenby[7], Ewart W. Kuijk[1], Annemieke Kavelaars[8], Cobi J. Heijnen[8], Lesley Regan[2], Jan J. Brosens[2]*, Nick S. Macklon[1,9]

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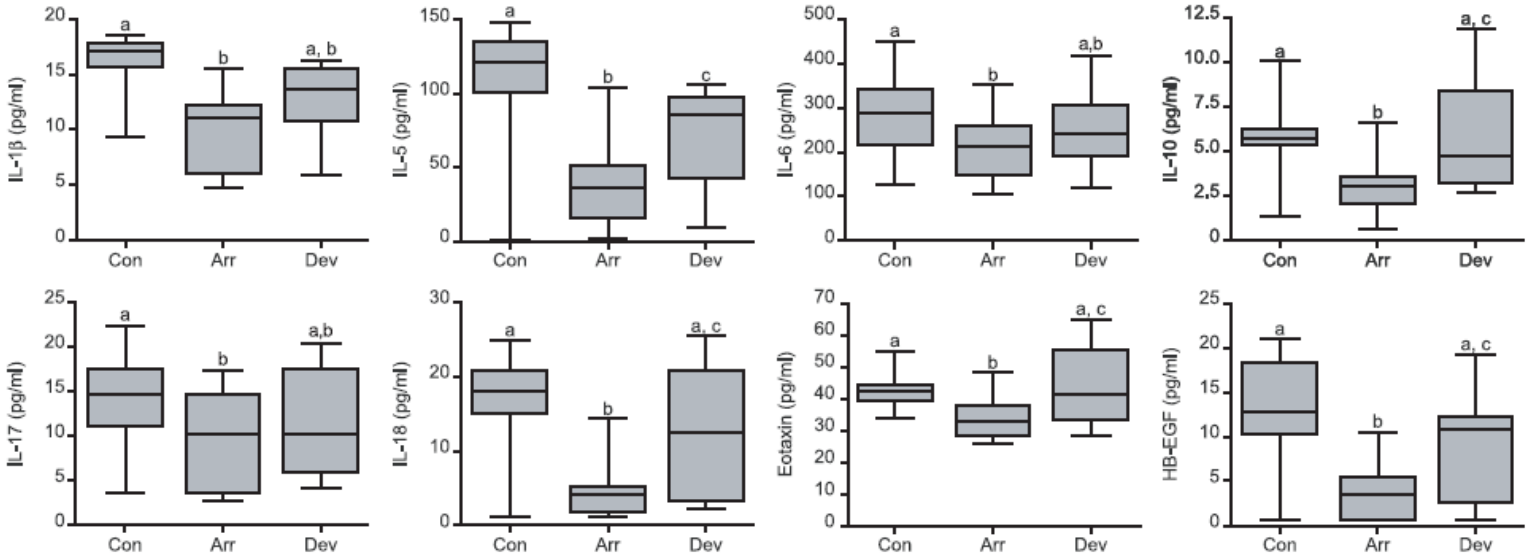
Decidualized ESCs selectively recognize the presence of a developmentally impaired embryo and respond by inhibiting the secretion of key implantation mediators (e.g. IL-1b and HB-EGF) and immunomodulators (e.g. IL-5, -6, -10, -11, -17, and eotaxin).



- Spontaneous decidualization of the endometrium resulting in cyclic menstruation and regeneration represents a strategy to facilitate implantation and at the same time to safeguard the mother against abnormal embryos.

- **As a result, the miscarriage can be seen as a natural process of quality control.**

# Decidual cell migration

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- Decidualizing endometrial stromal cells are programmed to migrate toward implantation-competent blastocysts, which is an integral step in the implantation process (Weimar CH, 2007; Grewal S, 2008 and 2010)

- This migration is triggered by signals from the trophoblast,
  - especially platelet-derived growth factor- AA (PDGF-AA) (Gellersen B, 2013; Gellersen B, 2010; Schwenke M, 2013; Weimar CH, 2013).

# Decidual cell migration

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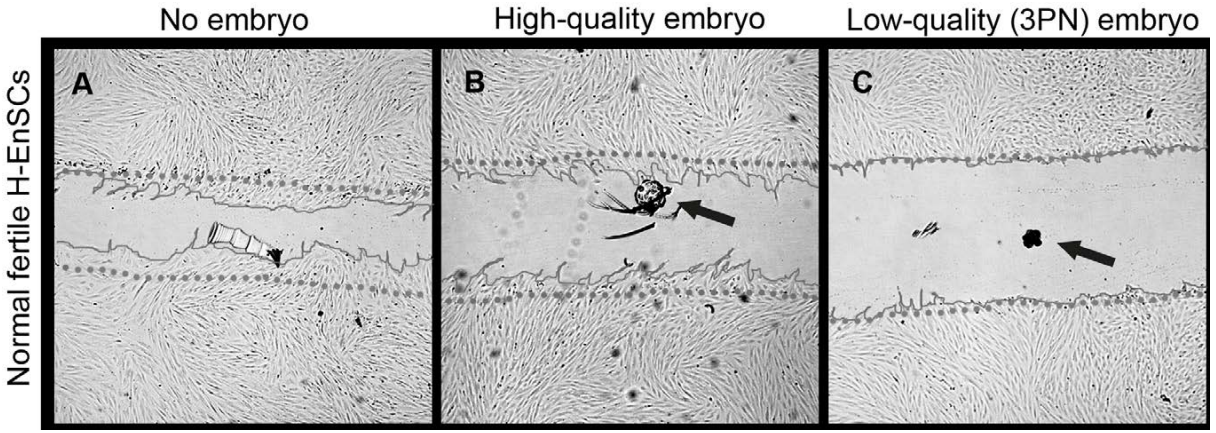
- Weimar et al. observed that normally decidual cells migrate toward to high-quality human embryos and entirely inhibited in the presence of chromosomally abnormal embryos.
  - decidual cells have the capacity to actively hinder invasion and outgrowth of abnormal human embryos



# Endometrial Stromal Cells of Women with Recurrent Miscarriage Fail to Discriminate between High- and Low-Quality Human Embryos

PLoS ONE, 2012

Charlotte H. E. Weimar[1], Annemieke Kavelaars[1], Jan J. Brosens[2], Birgit Gellersen[3], Johanna M. T. de Vreeden-Elbertse[4], Cobi J. Heijnen[1]*, Nick S. Macklon[4,5]

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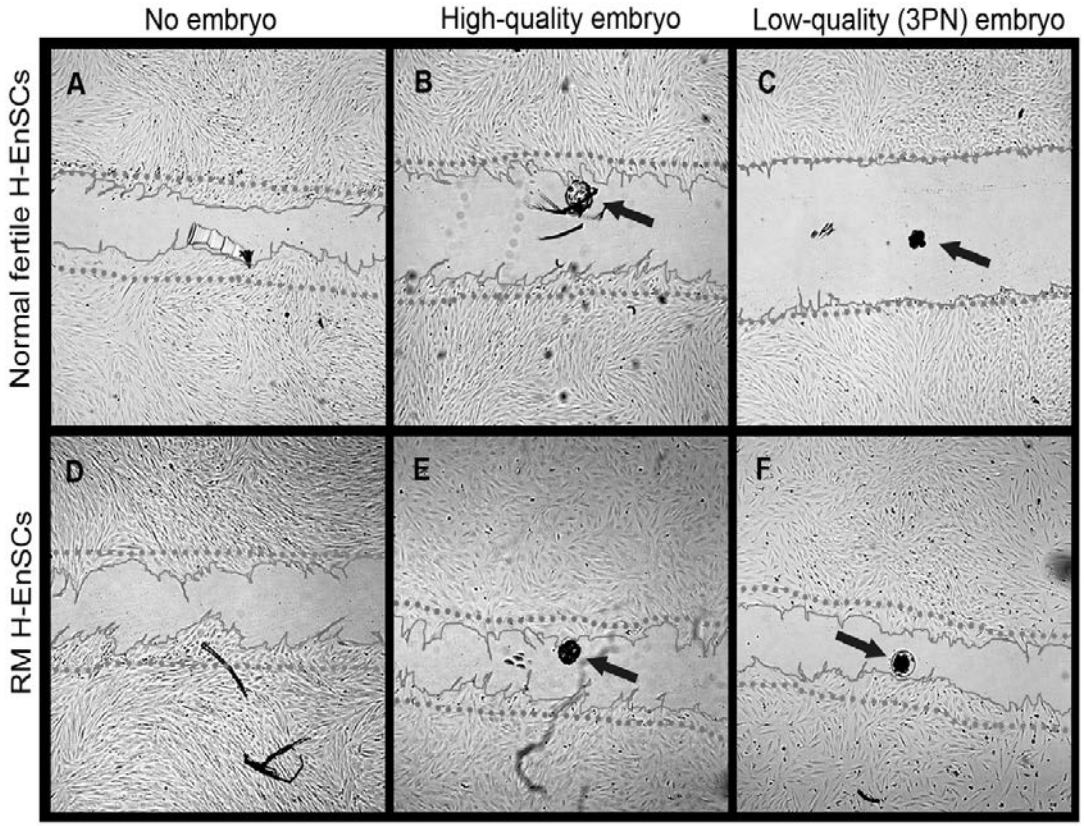


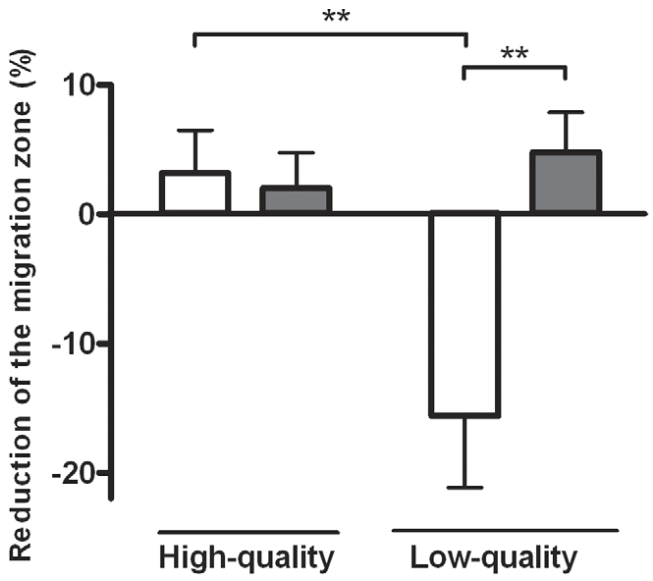

# Abnormal decidualization

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- Abnormal decidualization or so called “Super-receptive” endometrium has the **reducing ability to be ‘selective’ in response to embryo quality, so** embryos of low quality are implanted but rejected on a later stage, presenting a clinical miscarriage.

  Conversly,

- In some cases of decidual deregulation, endometrial biosensor **fail to respond to signals of high-quality embryos** and represent a suboptimal environment for implantation and development.
  - As a result, top quality blastocysts fail to implant.

  (Macklon N et al, 2014; Salker M et al, 2011)

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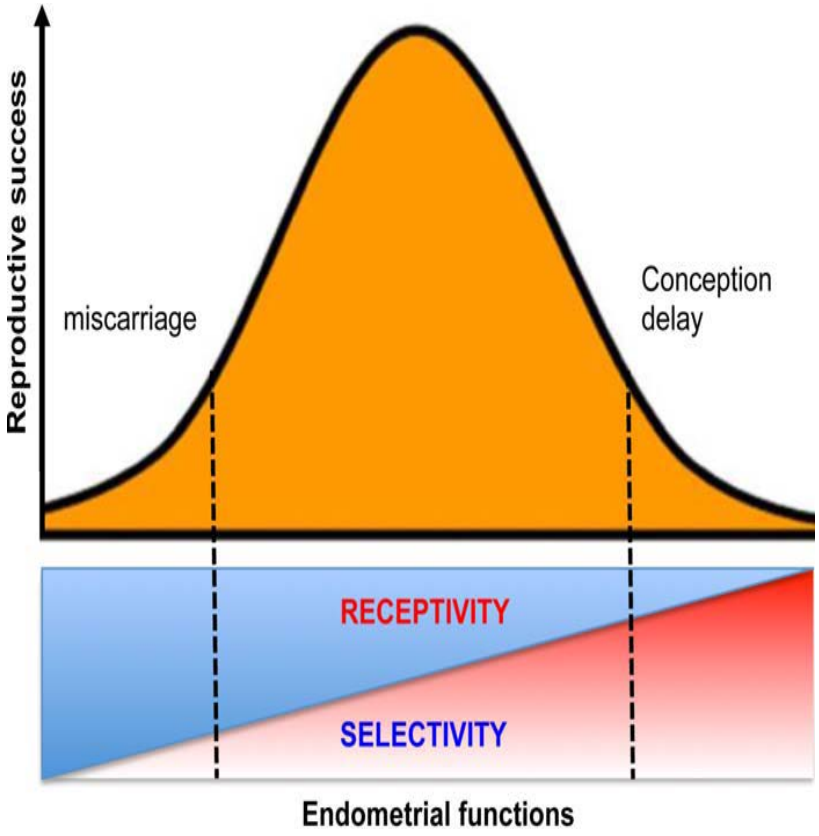
# Selective and receptive endometrium




Macklon and Brosens.
Biology of reproduction, 2014

A prolonged or unopposed receptive phenotype will lead to **miscarriages.**

Conversely,

Premature or excessive decidualization will increase the barrier function of the endometrium and super selectivity and **reduce the likelihood of pregnancy** or represent **recurrent implantation failure** (after IVF).

- Endometrial function can be considered in terms of both the epithelial feature of receptivity or ability to recognize and respond supportively to a high-quality embryo and the decidual function of selectivity.

- **The implication of this data is to reveal**

  **RM and recurrent implantation failure as two sides of one process.**

# Conclusions:

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- 1. True incidence of pregnancy loss (preclinical plus clinical) is closer to 50%, which renders miscarriage as the most common complication of pregnancy.

- 2. Women with recurrent miscarriage produce more aneuploid embryos than normal fertile women.

- 3. Diminished ovarian reserve may contribute to recurrent pregnancy loss and represent higher percentage of aneuploid blastocysts in age group under 38.

- 4. Miscarriage rates are positively correlated with sperm DNA damage levels with higher abortion rate in threshold of DNA fragmentation index > 27 %.

# Conclusions:

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- 6. Decidual cells have the capacity to abandon impaired embryos so the miscarriage can be seen as a natural process of quality control.
- 7. Impaired decidual programming of the endometrium is an important mechanism underlying miscarriage.
- 8. Recurrent miscarriage and Recurrent implantation failure are two sides of one process.

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# THANK YOU!